Biological and ecological processes during the establishment of a marine invasion: the *Siganus* rabbitfishes from the Red Sea to the coastal areas of Cyrenaica, Libya

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Acknowledgments

In The Name of Allah, the Most Beneficent, the Most Merciful

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Declaration

I declare that the work presented in this thesis my own, unless otherwise stated and have not been submitted for degree any other qualification at this or other university.

Abdulghani ABDULGHANI

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Chapter I

Biological invasions: definitions, implications, ecological and evolutionary consequences.

Abstract

The rabbitfishes *Siganus luridus* and *S. rivulatus* are two Lessepsian species that have invaded a large part of the Mediterranean Sea and offer a unique opportunity to examine ecological and morphological variation during the process of invasion and establishment.

Using and integrated geometric morphometric and stable isotope approach we investigated ecological adaptation in these two species from native into novel Southern Mediterranean habitats. A total of 490 fish were collected over two years (2014 and 2015), which showed a greater overlap in morphological variation in the novel habitat (Mediterranean Sea) compared to the native Red Sea. On the other hand, stable isotopes indicated that the invading populations segregated trophic niches more starkly than in the native habitat.

The introduction of genetic markers to infer population expansion uncovered a more marked decrease in genetic variation in *S. luridus* compared to S. rivulatus, during the process of invasion. Collectively, data contribute to reconstruct the jigsaw puzzle underpinning the success and ecological diversification of these coastal species, raising considerations for their management and that of the environment that changes around them.

Introduction

1.1 Biological invasions

1.1.1 Biological invasions: definitions

Many definitions have been applied to explain what “invasive species” are; in a number of them, the term invasive is associated with established species which are agents of change and threaten biological diversity or abundance of native species and the ecological stability (environmental impact) of invaded ecosystems (IUCN, 2002; EPA, 2001; Blackburn *et al.*, 2014). To understand invasion biology, a few terms must be explained. “Invasive species” is a broad label (Pereyra *et al.,* 2016) that can be used as a synonym for alien, non-native or exotic organisms. A species that has been transferred as a result of human activities over a geographical barrier of a native area to a new area is included in this definition (“introduced species”). Following Richardson *et al.* (2000), to analyse the introduction of a taxon, five definitions may be employed: 1) Established or naturalized, which is used to describe taxa that have the ability to reproduce and sustain a population in the new inhabited area; 2) Casual, which describes taxa that cannot reproduce and can be located sporadically; 3) Invasive, which is used for introductions that take place in great quantity and affect the native biodiversity negatively; 4) Pest, which refers to an introduction of a taxon not wanted by human beings in the area; 5) Transformer, which refers to an introduction where a taxon has the capability to make modifications in the functioning of the ecosystem. In this thesis, for consistence we will use the term invasive, following a combined definition, which consider spread, dominance and impact (Pereyra *et al.,* 2016): “A species is invasive because it spreads and has high population growth/dominates the invaded assemblage and produces negative impact".

To understand invasion ecology, it is also important to have adequate knowledge on the ecological processes in the systems being studied before the invasion. At the ecosystem level, species invasions have primarily negative effects on the native biodiversity (Streftaris and Zenetos, 2006; Galil, 2007; Lasram and Mouillot, 2009; Zenetos *et al.,* 2009). However, the field of invasion biology is not free from controversy, with studies also showing that invasive species may not have necessarily negative impacts on the biodiversity or the performance of the ecosystem. In some cases, it has been shown that a non-indigenous species can lead to an expansion in ecosystem functions, by adding new ecological roles, increasing the ecological traits present and resulting in an increase in functional redundancy (Reise *et al.,* 2006).

The invasion process can be separated into four/five phases, as described by Heger and Trepl (2003) and Blackburn *et al*. (2011). These phases are: the arrival phase where a taxon is introduced irrespective of the means used; this can be also separated in transport and introduction; the establishing phase where a taxon can reproduce in the different biotic and abiotic factors in the new habitat; the integration phase where the taxon is able to create new ecological connections in the new habitat; and finally the dispersal or spreading phase, where the taxon can expand its populations across the new habitat. A taxon that invades an area can at times reach a certain climax of density and then suddenly decrease, forming a path known as "boom and bust".

When an introduced species fails to establish itself in the new environment, it may be due to the lack of resources or to strong predatory pressures (Williamson and Fitter, 1996). Studies undertaken so far have mostly investigated the circumstances that facilitate these invasions and the biological consequences resulting from these intrusions. Most of the facilitators are usually physical such as an increase in temperatures of the sea water and a change in climate. Others are of a biological nature such as the characteristics of taxa and habitats that can be invaded. Biological consequences resulting from invasions determine economic (Streftaris and Zenetos, 2006) and biodiversity concerns, for example competition with the local key taxa (Golani, 1993, 1994; Azzurro *et al.,* 2007) or habitat modification (Kalogirou *et al.,* 2007) or the spread of new pathogens (Wallentinus and Nyberg, 2007; Bariche *et al.,* 2009). Invasive species often owe their success in colonizing new ecosystems to certain characteristics that make them more difficult to control and these characteristics include the capacity to thrive in different environments and tolerate a wide range of environmental conditions, high growth and reproduction rates, a lack of natural predators and an ability to exploit a variety of food sources (Otero *et al.,* 2013).

1.1.2. Biological invasions: implications

The spread and establishment of invasive species is a major concern for the conservation of ecosystems (Galil, 2007). In marine ecosystems, the introduction of invasive species can have major effects on the biodiversity, and thus effect of the structure and function of ecosystems. The understanding of potential effects following the introduction of invasive species requires a good knowledge of ecological processes in the ecosystems, such as diversity, structure, and function of natural communities. Invasive species are increasingly frequent, and

can have detrimental consequences, including the erosion of biodiversity and the disruption of invaded ecosystem function.

Biological invasions in marine habitats represent a recognized worldwide threat to the integrity of native communities in new habitats, leading to population losses and extinctions on a local scale (Ricciardi, 2004). The extent of the impact has been so severe that invasive species are regarded as the second biggest cause of biodiversity loss after habitat destruction. However, the effect of most invasive species in new habitats remains unknown, and the predictability of their direct and indirect impact remains unclear.

In general invasive species pose a threat to biodiversity by impacting native species and ecosystems (e.g., changing nutrient cycling; consuming or preying upon native species changing the whole ecosystem structure and functioning: competing or hybridizating with native species). Invasive species may induce habitat modification and alteration of ecological conditions and the unintentional introduction of toxic species, parasites and pathogens, may have an impact on both the ecosystem and human health (Byers *et al.*, 2010; Streftaris and Zenetos, 2006).

1.1.3. Ecological consequences of invasions

Ecological impacts of invasions can range from the displacement of native species as a result of niche overlap and competition for space and food, to a more direct effect where some introduced species prey on local wildlife (Olenin *et al.*, 2002). An example of invaders' impact on community-level has been reported by Crooks (1992) and Crooks (2002): the exotic Asian mussel *Musculista senhousia* had a drastic effect on the abundance and diversity of native species in mudflat communities in Mission Bay, San Diego. Also, Leonard et al. (1999) mentioned that the invader European green crab Carcinus maenas in the eastern USA, can substantially alter surrounding habitats through predation on mussels in areas of lower water flow than the areas with higher water flow, where green crabs were unable to forage in areas of higher flow, communities were dominated by mussels and their associated fauna. The Mediterranean Sea harbours around 986 introduced species representing 5.8 % of its known flora and fauna (Zenetos *et al.*, 2010, 2012). It can be considered as one of the regions most severely affected by marine species invasions. One example is the highly invasive alga *Caulerpa taxifolia*, which can occupy many shallow water habitats and alter their physical and chemical environmental conditions, seriously affecting coastal ecosystems, and also causing

severe economic impacts on fisheries (Boudouresque *et al.,* 1996). More than 200 invasive marine molluscs have been recorded off the Mediterranean coast. Most of them are of Indo-West Pacific origin and are believed to have entered the Mediterranean through the Suez Canal (Zenetos *et al.,* 2012). They display a distinct migration pattern beginning along the Mediterranean coast of Israel, moving north to the south coast of Turkey and Cyprus before entering the Aegean Sea and pushing westwards towards Malta, Italy and elsewhere.

Similarly, invasive alien crustaceans can have severe negative impacts on native ecosystems. They may completely change native communities through alteration of trophic interactions, interference, competition, disease transmission or habitat modification (Snyder and Evans, 2006). For example, the crab *Percnon gibbesi*, probably the most invasive decapod species found in the Mediterranean to date, has spread rapidly in the region, forming thriving populations in a very short space of time. Its feeding habits (it consumes primarily algae but also crabs and other crustaceans, polychaetes, gastropods and jellyfish) may affect the structure of benthic communities, particularly algal assemblages, and it may compete with native species for food and shelter (Katsanevakis *et al.,* 2011). In addition, eight species of invasive penaeid prawns have been recorded in the Mediterranean (Galil, 2007).

Studies have shown that these exotics compete intensively with native species over space and various resources, and this often lead to local displacement or elimination of the native species from the invaded area. The presence of the Lessepsian (from the Red Sea, see below) goldband goatfish *Upeneus moluccensis* resulted in the decline of the native *Mullus barbatus* in shallow waters. Similarly, the brushtooth lizardfish *Saurida undosquamis* invasion affected the native *Merluccius merluccius* and *Synodus saurus* presence in shallow waters. Today *M. barbatus* and *S. saurus* are found mainly in deeper waters along the Levantine coast (Golani and Ben Tuvia, 1995). Further studies indicated that the Lessepsian *Siganus rivulatus*, and probably *Siganus luridus* are replacing the native *Sarpa salpa* while the narrow-barred mackerel *Scomberomorus commerson* is replacing the native *Argyrosomus regius* off the Levant (Bariche *et al.,* 2004; Galil, 2007).

The native prawn *Melicertus kerathurus* has been outcompeted by various Lessepsian prawns in the Levant and off Turkish waters (Galil, 2007). The very common Lessepsian bivalves *Pinctada radiata*, *Chama pacifica* and *Spondylus spinosus* have replaced native Mediterranean bivalves. It is not clear whether *Portunus segnis*, which constitutes the only crab of substantial commercial interest along the Levantine coast, has outcompeted a native species

or not. The seasonal abundance of the Lessepsian jellyfish *Rhopilema nomadica* is certainly damaging fishery catches (feeding on larvae, clogging nets, damaging fishes in trawls and seines) and affecting the tourism sectors (venomous stings) in the eastern Mediterranean. The dramatic spread of other highly poisonous fishes such as the pufferfish *Lagocephalus sceleratus* along the Mediterranean coasts can also cause severe economic impacts on fisheries: their flesh is toxic when consumed and the fish damage both fishing gear (long lines, entangling nets) with its strong teeth. Other common Lessepsian species, with commercial importance or not, are probably engaged in intense competition with (or have already outcompeted) native species, and are thus causing an unknown damage to the Mediterranean fishery and environment.

1.1.4. Evolutionary consequences of invasions

Invasive species evolve in response to new environmental factors in new habitats and in response to their interactions with native species. Recent studies have shown that invaders can rapidly adapt to the new environments in which they find themselves (e.g., Huey *et al.,* 2000). The knowledge about consequences of individual genetic changes in invasive species is still poor. Ferrero *et al.* (2015) pointed out that the features of successful invasive species are the ability to invade a wide variety of habitats, without genetic variation between them. While it is not surprising that an invasive species would evolve in its new habitat in response to a new set of selective pressures, changes in its genetic structure might also happen if successful mating with closely related species occur. These genetic changes are related to hybridization (mating between two different species or two genetically distinct populations) and introgression (the incorporation of genes from one species or population to another through hybridization that results in fertile offspring that further hybridize with parental populations or species, “backcross”). Over several generations, introgression can result in a complex mixture of parental genes, while in simple hybridization 50% of genes will come from each of the two parental species.

Studies of genetic diversity and the potential for rapid evolution of invasive species may provide useful insights into what allow species to become invasive. More information about the genetics and evolution of invasive species or native species in invaded communities, as well as their interactions, may lead to predictions of the relative susceptibility of ecosystems

to invasion, identification of potential future key alien species, and predictions of the subsequent effects of removal.

The evolutionary genetics of invasive species could offer insights into mechanisms of invasions. Recent studies suggest that the invasion success of many species might depend more heavily on their ability to respond to natural selection than on broad physiological tolerance or plasticity (Bucciarelli *et al.,* 2002; Karako *et al.,* 2002; Bonhomme *et al.,* 2003; Hassan *et al.,* 2003; Azzurro *et al.,* 2007; Terranova *et al.,* 2006; Iannotta *et al.,* 2007). Thus, these studies stress the importance of genetic architecture, selection upon which could result in evolutionary adaptations and possibly speciation.

Invasive species faces various pressures when inhabiting new environment and according to the fundamental principles associated to population genetics, the genetic variability of these invasive species will determine how capable they are in adapting to the environment. In any biological invasion, many invaders will often go through variable time periods from the initial colonization to the successive population advance and expansion. Holt and colleagues (2005), claims that this variable time periods have diverse ecological and demographic effects, or it may be determined by the needed time for the evolutionary adaption to the new environment.

Finally, there has been a wealth of studies in recent years examining genomics and adaptation of invaders. For example, Bock *et al.* (2014) suggested that the rapid adaptation is not linked by genetic variation in an invasive species, also as they pointed out that population bottlenecks and genetic drift typically have negative effects on invasion success. Also, Dlugosch *et al.* (2015) suggested to link the genetic structure and comparative demographic analyses to study genetic changes and evolution of invasive species. The scale of processes invasion has genetic consequences as the genetic variation can affect of successful establishment. Kinnison and Hairston (2007) and Gaston (2009) reported that the knowledge of the population-level consequences of individual genetic changes is still poor. Keller *et al.* (2014) reported that the assessing genetic consequences for the invasive species depends the occurrence of multiple invasive species and admixture. Dlugosch *et al.,* (2015) explanied talso hat the ecology and demography of populations should be taken into account when interpreting patterns of genetic diversity in invasive species.

1.1.5. Mediterranean biodiversity

The term biodiversity refers not only to the number of species living in an area, but more broadly includes genetic variability and species interactions. The Convention on Biological Diversity defines biodiversity as "the variability among living organisms from all sources and ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (www.biodiv.org).

Coll and Colleagues (2010) have recently estimated the number of species in the Mediterranean Sea in the order of 17,000. Their list includes13.2% crustaceans, 12.4% mollusks, 6.6% annelids, 5.9% platyhelminthes, 4.5% cnidarians, 4.1% vertebrates, 4.0% sponges, 2.3% bryozoans, 1.3% tunicates and 0.9% echinodems. The remaining species are composed by other invertebrate groups (14%), plants (5%) and prokaryotes (26%).The Mediterranean contributes to a substantial proportion of biodiversity, corresponding to somewhat around 25% of world marine species, which is a conspicuous value if one considers that the Mediterranean Sea corresponds only to the 0.82% in surface area (Bianchi and Morri, 2000; Coll *et al.,* 2010) and 0.32% in volume of the world oceans (Rilov and Galil, 2009). However, Bas *et al.* (1985) explain that the Mediterranean, despite its great diversity, has very few dominant species. Flora and fauna found in the Mediterranean have undergone millions years of evolution that have turned the Mediterranean Sea into a unique mixture of subtropical and temperate elements, and a large proportion of endemic species (Zenetos *et al.,* 2009).

The Mediterranean Sea biota is constituted primarily of Atlantic-Mediterranean species which make up 62.2% of the total number of species, obtained from adjacent biogeographic provinces in the Atlantic Ocean, beyond the strait of Gibraltar. It is also noteworthy that a large part of species in the Mediterranean are endemic (21.95%), while others are cosmopolitan, being either Indo-pacific (5%) or circumtropical (13%; Coll *et al.,* 2010). There are differences in proportions among the main taxonomic groups, as well as among the different regions of the Mediterranean Sea. Currently, the Mediterranean biodiversity is undergoing fast change, caused of climate change and human activities.

Since the opening of the Suez shipping Canal in 1869, the Mediterranean Sea is experiencing a significant influx of Red Sea species, a phenomenon known as 'Lessepsian migration' in recognition of Ferdinand de Lesseps, the French diplomat who developed the Suez Canal. During the last ten decades, at least 300 Indo-pacific marine animal species penetrated the Mediterranean Sea (Por, 1978; Boudouresque, 1999; Galil, 2000). These

introductions have produced important changes in species composition of Mediterranean communities and have resulted in mixed Red Sea-Mediterranean communities (Fishelson, 2000; Galil, 2000). Lessepsian species now acclimated in the Mediterranean include algae, sea grasses, various invertebrates and fish (Golani, 1998).

Rilov and Galil (2009) discuss the history, distribution and ecology of marine bioinvasions in the Mediterranean. The authors divided the Mediterranean into three regions: western, central and eastern and described a gradient of increasing species diversity toward the east from the west in the Mediterranean: among all major groups of plants and animals, the number of species tends to be lower toward western Mediterranean as compared to the eastern and central parts of sea (Fig. 1).

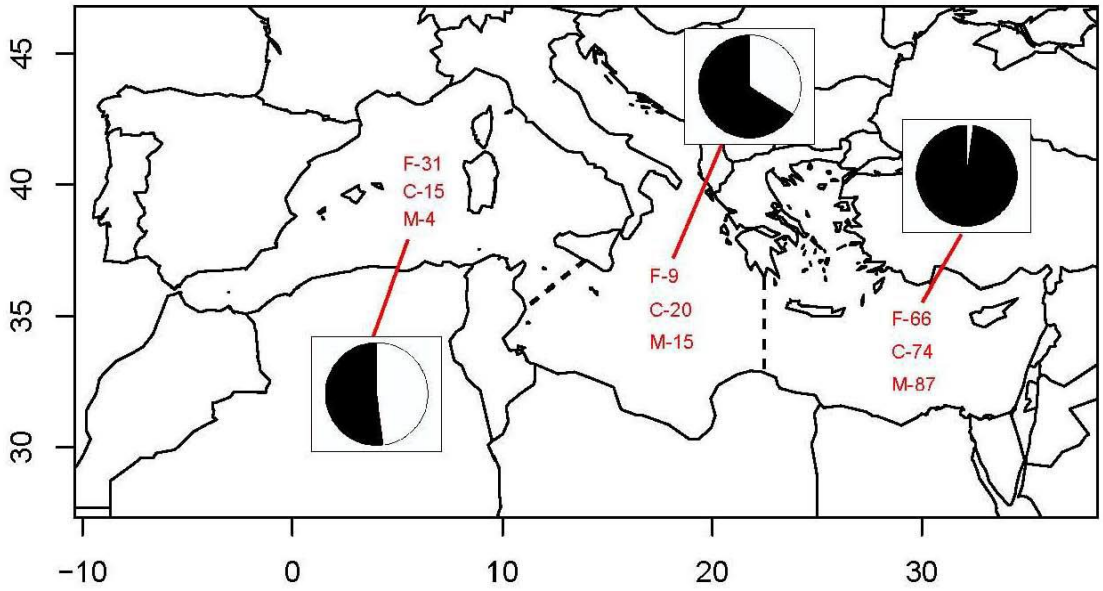


Figure 1. Spatial patterns of invasive species in the Mediterranean. Numbers indicate the percentage of each taxa (F: Fish; C: Crustacea; M: Mollusca) in each region of the Mediterranean (please note that the sum of the percentages is more than 100% as some species occur in more than one region). Pie charts indicate the percentage (all taxa pooled) with either Pacific (black) or Atlantic (white) origin. From Rilov and Galil (2009).

According to Ketchum (1983), the littoral and benthic populations reveal a similar change in the diversity of species as well as numbers, with a notable decrease towards the east from west, and toward south from the northern Adriatic. There are differences in proportions among the main taxonomic groups, as well as among the different regions of the Mediterranean Sea. Currently, Mediterranean biodiversity is undergoing fast change, caused of climate change and human activities.

1.1.6. Biological invasions in the Mediterranean Sea

These introductions have produced important changes in species composition of Mediterranean communities and have resulted in mixed Red Sea-Mediterranean communities (Fishelson, 2000; Galil, 2000). The Suez Canal has become a link between biotas: it links the tropical Red Sea (rich in bio-diversity) with the semi-tropical Eastern Mediterranean (poor in bio-diversity). The Mediterranean Sea is presently believed to be the main hotspot on earth for bio-incursions (Rilov and Galil, 2009) and it is the main receiver of invasive species across a variety of taxa, from macrophytes to invertebrates and fish (Streftaris and Zenetos, 2006). Invasion in the Mediterranean Sea has been increasing in recent years and this has had serious economic, social, and ecological consequences (Streftaris and Zenetos, 2006). There is, however, no comprehensive documentation of the true scale of biological impacts caused by the invasive species that have been introduced via the Suez Canal, making it difficult to generalize and devise mitigating strategies (Rilov and Galil, 2009). However, it can be argued that some invasive taxa have had very strong biological impacts (Kalogirou *et al.*, 2007; Bariche *et al.*, 2009).

According to Galil (2009), the opening of the Suez Canal resulted to a great influx of hundreds of organisms which gained passage from the Red Sea. Golani (2010) explains that the Lessepsian migration had profound effects on the Mediterranean biota due to the displacement of native fish species by competing invaders. However, due to the fact that no extinction has been recorded thus far at the basin level, this increase in new species has contributed to an increase in diversity of species dwelling at the regional level. In order to assess and manage the increasing impacts of Lessepsian invasions on Mediterranean biotas, further investigations are needed on the process of establishment of key invaders. According to updated checklists presented by Zenetos *et al.* (2010, 2012), a total of 986 invasive species were known in the Mediterranean as of December 2012. Based on the Mediterranean Action Plan for invasive species, the main known pathway/vector of species introduction into the Mediterranean Sea is indeed the Suez Canal followed by shipping and aquaculture (both marine and brackish species) and trade in live marine species (UNEP-MAP-RAC/SPA, 2005).

Marine invasive species are regarded as one of the main causes of biodiversity loss in the Mediterranean. There were 48 new entries since 2011 which can be interpreted as approximately one new entry every two weeks (Galil, 2007; Coll *et al.*, 2010). More than 5% of the marine species in the Mediterranean are now considered non-native species (Zenetos *et*

al., 2012). According to the latest regional reviews, 13.5% of those species are classed as being invasive in nature, with macrophytes (macroalgae and seagrasses) the dominant group in the western Mediterranean and Adriatic Sea (Fig. 2), and polychaetes, crustaceans, ascidians and ctenophores (Fig. 3), molluscs (Fig. 4) and fishes (Fig. 5) in the eastern and central Mediterranean (Galil, 2009; Zenetos *et al.*, 2010, 2012).

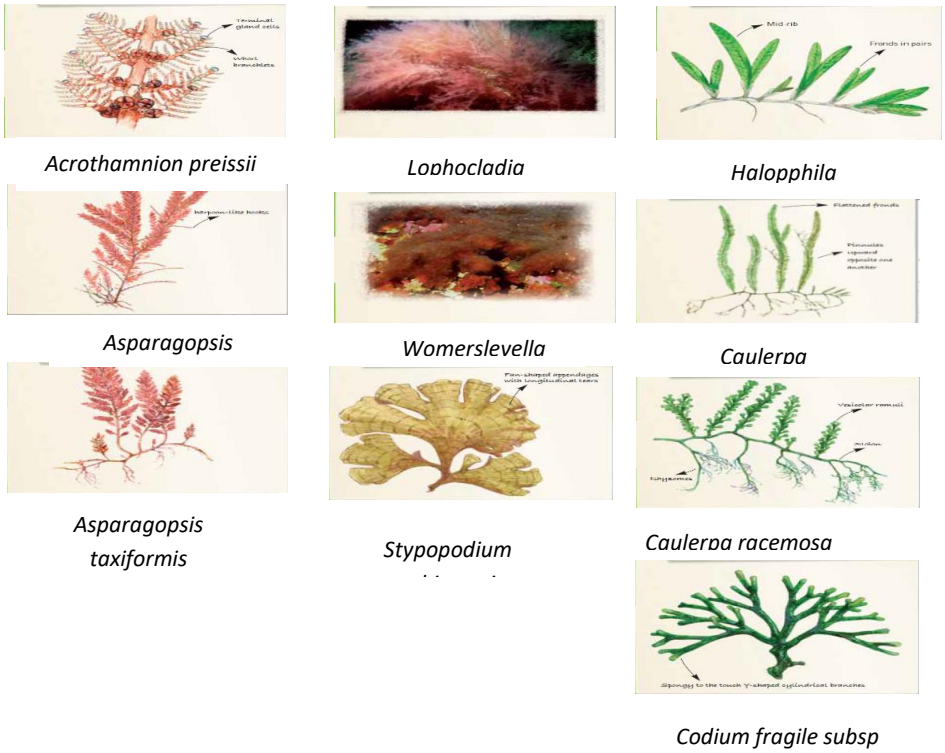
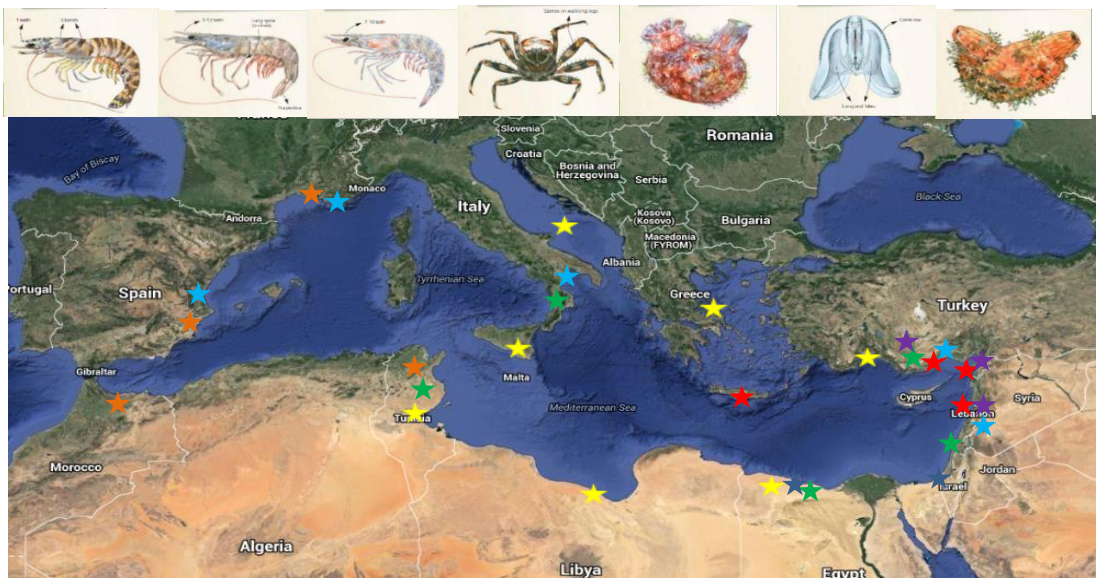
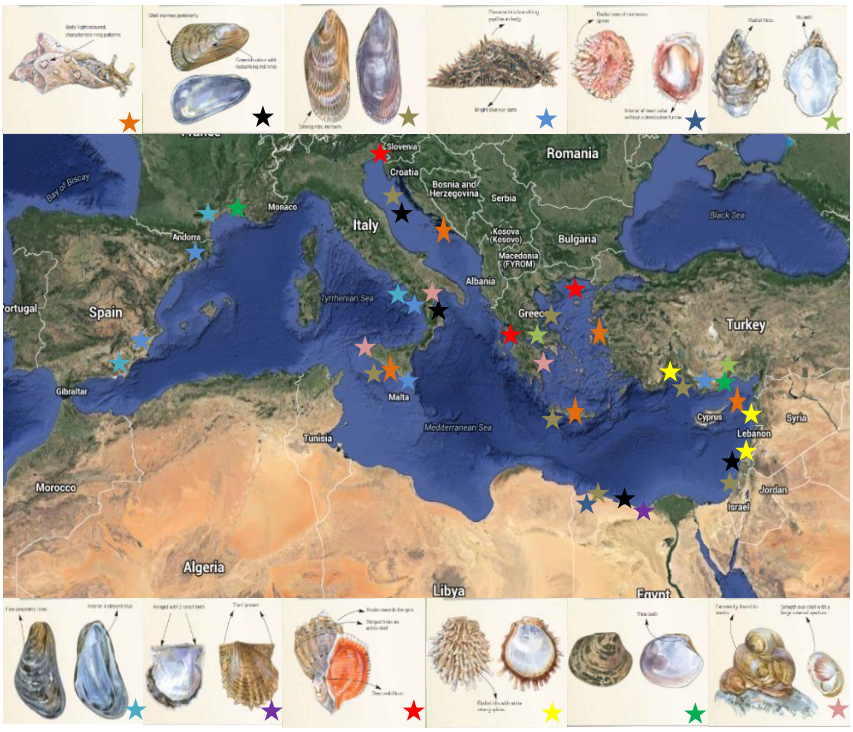


Figure 2. Selected invasive algae in the Mediterranean (from Otero *et al.*, 2013).



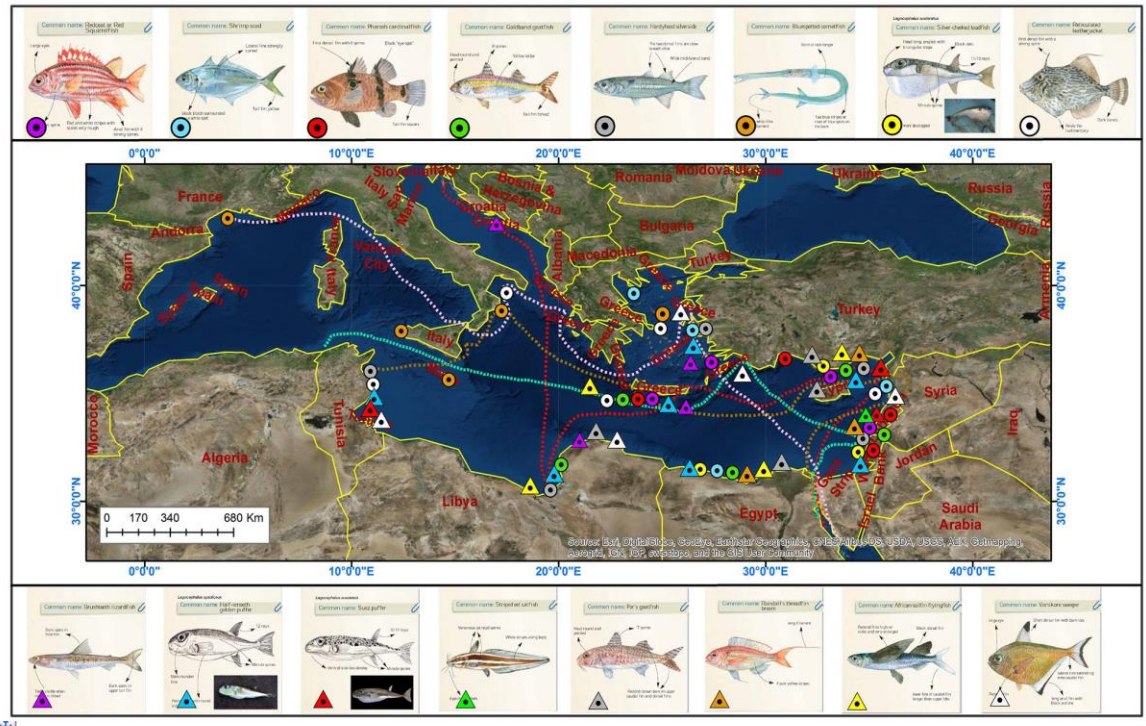
Marsupenaeus japonicus, *Metapenaeus monoceros*, *Metapenaeus stebbingi*, *Percnon gibbesi*, *Herdmania momus*, *Mnemiopsis leidyi*, *Microcosmus squamiger*.

Figure 3. Patterns of spread of invasive crustacean, ctenophoran and ascidian species in the Mediterranean (from Otero *et al.*, 2013).



Aplysia dactylomela ,*Arcuatula senhousia*, *Brachidontes pharaonic*, *Bursatella leachii*, *Chama pacifica*, *Crassostrea gigas*, *Crepidula fornicate*, *Limnoperna secures*, *Pinctada imbricate radiata*, *Rapana venosa*, *Spondylus spinosus*, *Venerupis philippinarum*

Figure 4. Patterns of spread of invasive molluscs species in the Mediterranean (from Otero *et al.*, 2013).



Scientific name	First record	Distribution	References
Sargocentron rubrum	Palestine 1947	Lebanon, Cyprus, Greece, Turkey	(Colaniand Ben-Tuvia,1985)
Atherinomorus forskalii	Egypt 1902	Palestine, Turkey, Lebanon, Greece, Libya, Tunisia	(Kimura *et al.*,2007)
Fistularia commersonii	Palestine 2000	Augean sea, Greece, Italy, Sicily, Malta and French	(Azzurro *et al.*, 2012)
Nemipterus randalli	Palestine 2005	Lebanon, Egypt and Turkey	(Bariche,2012)
Parexocoetus mento	Palestine 1935	Aegean sea, Syria, Egypt, Libya Albania and Tunisia	(Bariche, 2012)
Upeneus moluccensis	Palestine 1947	Lebanon, Syria, Turkey ,Rhodes, Egypt, Cyprus and Libya	Ismen , 2006)
Upeneus pori	Turkey 1950	Palestine, Lebanon, Syria, Turkey , Egypt, Cyprus and Libya	(Ismen , 2006)
Lagocephalus sceleratus	Turkey 2003	Lebanon, Egypt, Turkey	Nader *et al.*,2012)
Lagocephalus spadiceus	Turkey 1930	Turkey, Palestine, Tunisia	Nader *et al.*,2012)
Lagocephalus suezensis	Turkey 2003	Palestine, Turkey, Egypt, Libya, Tunisia	(Nader *et al.*,2012)
Plotusus lineatus	Palestine 2002	Palestine	(Edelist *et al.*, 2012)
Pempherisvani colensis	Lebanon 1979	Palestine, Lebanon, Greece, Turkey, Egypt, Libya, Tunisia	(Golani and Diamant,1991)
Saurida undosquamis	Palestine 1952	Cyprus, Turkey, Greece, Libya, Croatia	(Golani, 1993)
Alepe sdiedabe	Palestine 1927	Aegean sea, Egypt, Greece,	(Carpenter *et al* 1997)
Apogonichthyoides pharaonis	Palestine 1947	Cyprus, Turkey, Palestine ,	(Zenetes *et al.*, 2010)
Stephano lepis	Palestine 1927	Syria, Cyprus, Tunisia, Italy, Greece,	

Figure 5. Patterns of spread of invasive fish species in the Mediterranean. Data include scientific name, first record and the distribution for each species in the Mediterranean Sea.

The Suez Canal has supplied the largest number of successfully established aliens in the Mediterranean Sea in terms of magnitude, frequency and duration of transfer. For decades, the ongoing migration of marine species through the Suez Canal has helped to explain the richness of Red Sea invasive species in the eastern Mediterranean Sea, particularly in the Levant area (the south to Palestine, Lebanon and Syria in the east).

1.1.7. Biology and behaviour of invasive fish species

The oceans and seas are tremendously diverse and species-rich. They are home of countless organisms living in very different ecosystems. There are over 30,000 fish species in the world. Each of these fish types is part of a habitat, an ecosystem, and exists in complex interdependence with many other species in a food web. Fish are a vital component of marine habitats.

The Mediterranean Sea is inhabited by about 716 species of fish (Froese and Pauly, 2011); 80 are invasive and originate from the Indo-Pacific and the Red Sea (Cicek and Bilecenoglu, 2009; Bariche, 2010; EastMed, 2010; Golani, 2010). According to Simberloff and Rejmánek (2011), some of these immigrants became more dominant in the new environment, both numerically and ecologically, with overwhelming ecological impacts and large economic costs. Others, on the other hand, were unable to thrive in the new environment. The prediction of the species that will fall in each of the categories is an urgent but daunting task for ecologists, so much so that some have considered it the final goal of invasion biology (Kolar and Lodge, 2001). Numerous attempts have been made at seeking accurate prediction methods of the features of the invaders, as well as the invaded ecosystems (Catford *et al.*, 2009). However, so far few studies have revealed traits linked to invasiveness, and the data requirement for those studies require the gathering of multiple data, such life history data, network structure and phylogeny (Olden and Rooney, 2006; Schaefer *et al.*, 2011; Park and Potter, 2013).

Basing emphasis on the limited similarity hypothesis (MacArthur and Levins, 1967), there is a prediction of lesser opportunity of becoming abundant, for species that share similarities to others, as a result of greater interspecific competition. Recent theories regarding invasions agree that these differences which are understood as taxonomic, functional, or ecological in nature, make it possible for invaders to utilize unexploited niche opportunities to keep away from direct competition with inhabitant species (Shea and Chesson, 2002). There are other hypotheses, such as the biotic resistance hypothesis and the empty niche hypothesis (Mack *et al.*, 2000) which integrate the central relationships between niche parallels, and competition. Ergo, a central theme emerges within these theories, whereby a concept of commonality in the acceptance of niche space within the recipient communities, as a main factor influencing the likelihood of success for the newcomers (Olden *et al.*, 2003).

Only two Lessepsian species, *Fistularia commersonii* and *Siganus luridus* have so far been recorded along the French coast in the north-western Mediterranean, the farthest point from the

Suez Canal. These two species display different patterns of colonization: *F. commersonii* (see Fig.5 orange circle), widely distributed in the Indo-Pacific and eastern-central Pacific, was first recorded in the Mediterranean in January 2000 along the coast of Israel. Since then, the species has quickly spread throughout, reaching France in just 6 years. *Siganus luridus* instead (see Fig. 8 below, violet circle), usually found in the western Indian Ocean and Red Sea was first recorded in the Mediterranean in 1956 and progressively continued its geographical expansion through the eastern basin. It reached north-eastern Tunisia around 1970, but crossed the Strait of Sicily only in 2004; it reached the French coast in 2008 (Daniel *et al.*, 2009).

1.2. Siganidae

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Siganidae
Genus: *Siganus*
Species: *S. luridus* and *S. rivulatus*
(http://www.fishbase.org)

The *Siganus* fishes, or 'rabbitfishes', are small-sized and short-lived (4-6 years) and belong to the family Siganidae (Shakman *et al.*, 2008). The members of this family have a wide geographical distribution in the tropical and subtropical areas. Four *Siganus* species live in the Red Sea: *S. luridus, S. rivulatus, S. stellatus* and *S. argenteus* and two of them, *S. rivulatus* and *S. luridus*, invaded the Mediterranean Sea through the Suez Canal and have established themselves in the eastern Mediterranean Sea (Ben-Tuvia, 1966). They are now spread on the Egyptian, Palestinian, Turkish, Greek, Cypriot, Italian, Tunisian, and Libyan coasts. It is clear from the geographical distribution of the Siganidae that they have succeeded in migrating from the Red sea environment with high salinity and specific food availability to the east and west of the Mediterranean which has different salinity and food availability.

Rabbitfish are herbivorous and feed by nibbling the marine vegetation, usually grazing in schools with head directed downwards, so they live mainly around reefs and weedy areas. Their bodies are compressed, oval in outline and covered with minute scales that are smooth in touch.

The small mouth is equipped with a row of close-set teeth in each jaw. They have poisonous dorsal and anal spines for armour. The mucus covering the spines is toxic and although not fatal to man (Woodland, 1983) can produce unpleasant swelling and pain. For this reason they should be handled with care. *Siganus* fishes have important commercial value; most species are appreciated for consumption and fetch medium to high prices in the markets (Shakman and Kinzelbach, 2007).

1.2.1. *Siganus luridus* (Dusky spinefoot)

The body is deep, ellipsoid, and compressed (Fig. 6); the scales are small and embedded in the skin. Total length is commonly 5–20 cm. The dorsal fin (13–14 spines and 10 soft rays) begins above the pectoral fin base. The pelvic fin begins behind the pectoral fin base; the anal fin has 7 spines and 9 soft rays. The mouth is small with distinct lips. The maxilla does not reach the vertical plane through the eye and the incisor teeth are in a single row. The colour is dark brown to olive green with a touch of yellow on the fins, but varies regionally. At night, the colour is very mottled. It feeds on a wide range of benthic algae, mainly coarse brown algae, but seagrasses are also reported in its diet. *Siganus luridus* feeds at more or less the same rate in all seasons.

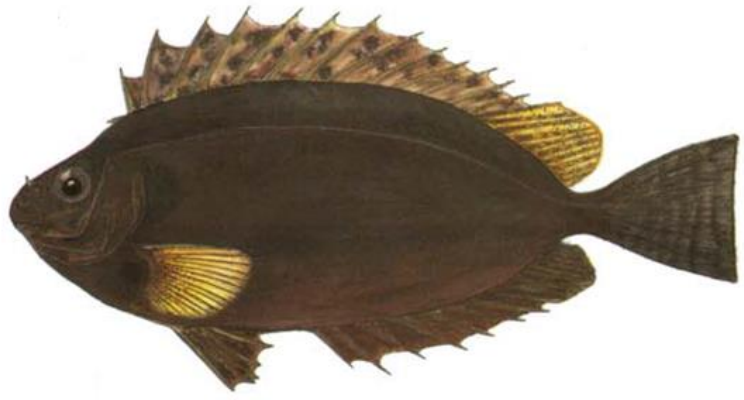


Figure 6. *Siganus luridus* (FAO; 1983)

The dusky spinefoot is a species usually found in the western Indian Ocean and Red Sea. It was first recorded in the Mediterranean in 1956 along the Palestine coast and progressively continued its geographical expansion through the eastern Mediterranean. It is widely distributed nowadays in the central and western parts of the Mediterranean and was first

recorded in Tobruq, Libya in 1970 (Stirn, 1970; Shakman *et al.*, 2008) and in Tunisia in 1971 (Ktari and Bouhalal 1971; Ktari and Ktari, 1974). Recently a newly settled population has been recorded on the Italian island of Linosa (Azzurro and Andaloro 2004). In 2008, two specimens were caught along the French Mediterranean coast at a depth of about 5-10 m in a site mostly characterized by rocky bottoms mixed with *Posidonia oceanica* beds. In 2010, it was also recorded in two different localities in the Adriatic Sea (Gulf of Trieste and southern Adriatic Sea, island of Mljet). Several specimens were also observed and photographed in France close to the Italian border between November 2011 and July 2012.

1.2.2. *Siganus rivulatus* (Marbled spinefoot)

This medium-sized fish has an ellipsoidal, compressed body (Fig. 7) covered with small scales embedded within the skin. It grows to a length of 5–25 cm. The dorsal fin has 13–14 spines and 10 soft rays. Key taxonomical features for this species' identification are pelvic fins with two stout spines connected by a membrane to the abdomen, and the forked tail fin. The mouth is small with distinct lips. Body colour is brown to grey green, and light-brown to yellow on the belly. There are fine, often faint, yellow-gold stripes on the lower half of body.

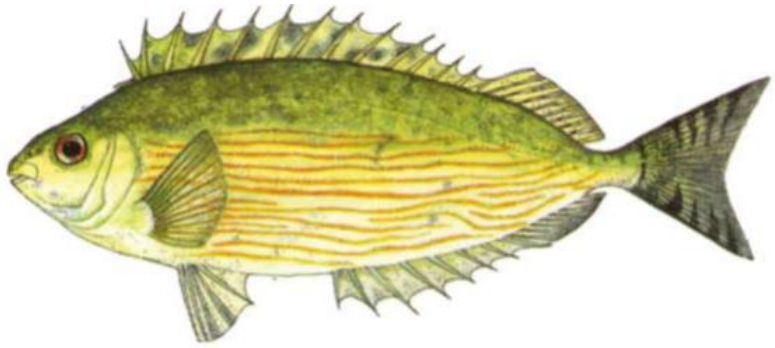


Figure 7. *Siganus rivulatus* (FAO; 1983)

The marbled spinefoot lives in shallow waters, preferring hard bottoms of compacted sand with rock, usually covered with vegetation. Adults live in small groups of 50 to several hundred individuals, feeding mainly green and red algae, such as *Ulva* spp. and *Hypnea* spp., and seagrasses (*P. oceanica*). The marbled spinefoot is a species usually found in the western Indian Ocean and Red Sea. It was first recorded in the Mediterranean in 1927 (Shakman *et al.*, 2008) along the Palestinian coast and spread progressively through the eastern Mediterranean:

Syria, Cyprus, the Aegean Sea, Libya, Tunisia, the Ionian Sea and the southern Adriatic (Croatia). *Siganus rivulatus* has not yet been reported from the western Mediterranean; however, its presence has been suspected in Corsica since 2010. Although erroneously reported in the literature, *S. rivulatus* does not appear to have established in Maltese waters (Schembri *et al.*, 2012).

1.2.3. Implication of *Siganus* species invasions

After the initial spread in the eastern Mediterranean, both *Siganus* species have been recorded by many authors all around the Mediterranean basin (George *et al.*, 1964; Kavallakis, 1968; Demetropoulos and Neocleous, 1969; Sala *et al.*, 2011; Fig. 8).

They strongly interact and compete with native herbivorous fish species with whom they share a common habitat and diet, including *Sarpa salpa* (Sparidae) and *Sparisoma cretense* (Scaridae) (Shakman and Kinzelbach, 2007) through competition for food resources (their diets overlap considerably) and habitat. The grazing pressure by both fish populations has severely reduced the composition and biomass of algal assemblages (Sala *et al.*, 2011), creating and maintaining underwater barren grounds composed solely of bare rock and patches of crustose coralline algae.

The spawning season lasts from May to September. Barich *et al.*, (2002) reported that spawning occurred in June in *S. rivulatus* and from May to July in *S. luridus*, but various studies have reported a spawning season ranging from 2 to 7 months for the two siganids in the Red and Mediterranean Sea (Popper and Gundermann, 1975; Popper *et al.*, 1979; Amin, 1985; Hussein, 1986; Saad and Sabour, 2001). A second spawning in September (George, 1972) or an extension of the first spawning to November (Mouneimné, 1978) was suggested for *S. rivulatus* in the Mediterranean Sea as young-of-the-year were found in the autumn. Water temperature plays a role in the determination of the timing and duration of spawning season (Lam and Soh, 1975; Popper *et al.*, 1976; Amin, 1985).

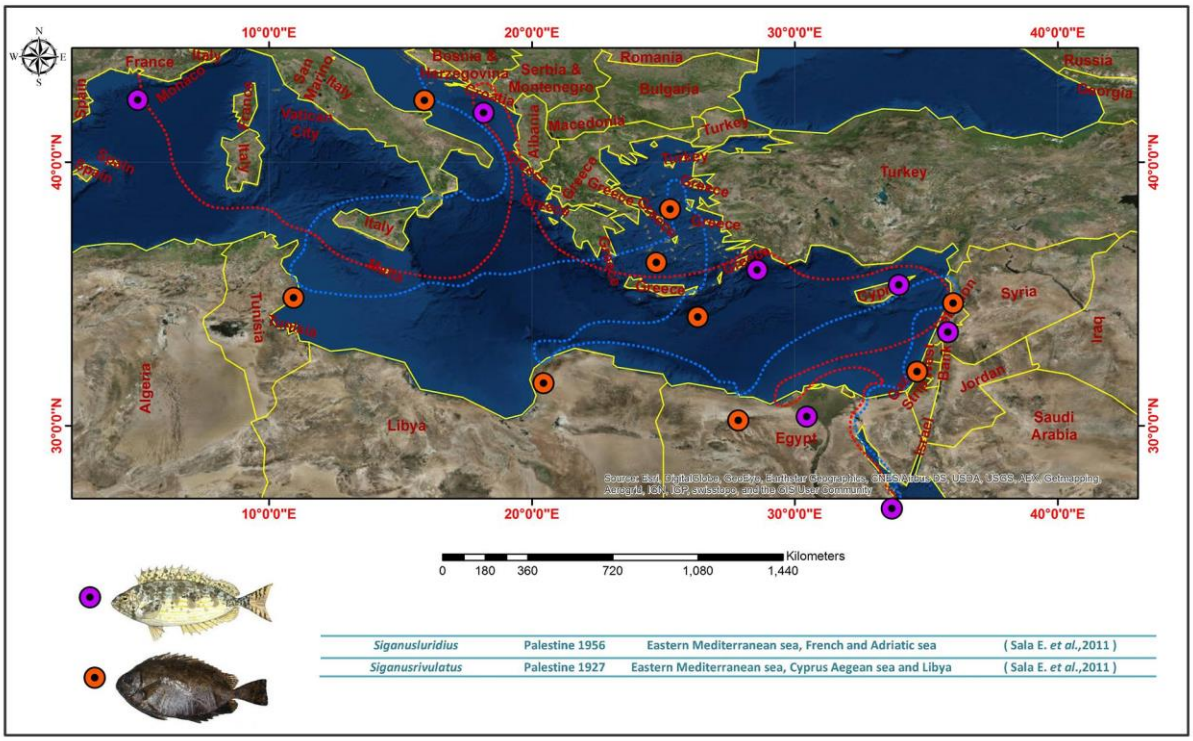


Figure 8. Location map of the records of *Siganus luridus* (orange dot) and *S. rivulatus* (violet dot) along the Mediterranean coast.

In the eastern Mediterranean Sea, seawater temperature is higher in summer and lower in winter than in the Red Sea. High temperature in summer appeared to be a limiting factor in the gonadal development of both siganid species, which have reduced their breeding seasons on the Lebanese coast compared to their native Red Sea: Popper and Gundermann (1975) noted that both invaders shortened their spawning season in the new environment.

There have been attempts to breed *S. rivulatus* in different areas (Ben-Tuvia *et al.*, 1973, Popper *et al.*, 1973, 1979). In the Jeddah region (Saudi Arabia), the marbled spinefoot reaches 200 to 300 g body weight after nine months feeding in cages (Thebaity *et al.*, 1984). Its reproduction was intensively studied in the Red Sea (Popper *et al.*,1979; Hashem, 1983; Amin 1985) and also in the eastern Mediterranean (George, 1972; Hussein, 1986; Saad and Sabour, 2001; Bariche , 2010). There are a few studies on the reproduction of *S. luridus* in the Red Sea (Popper and Gundermann, 1975, Golani, 1998) and a few studies also in the eastern Mediterranean (Mouneimné, 1978; Golani, 1993; Bariche , 2010).

Both species are distributed and established along the Libyan coast and they have become of commercial value, especially in the western part of the Libya and the Gulf of Sirte (Golani,

2002; Bilecenoglu and Taskavak, 2002; Torcu and Mater, 2000; Shakman and Kinzelbach, 2007). Despite their commercial exploitation, information on the biology and population structure of these species is scarce. Therefore, the present work aims to compare some biological aspects and the genetic composition of the rabbit fishes *S. luridus* and *S. rivulatus* harvested from the natural fisheries of the Cyrenaican coast in Libya and Red Sea in Egypt.

1.3. Aims of the study

The main goal of this study is to conduct an interdisciplinary investigation on the ecology of two, now common, conspicuous components of coastal biodiversity in Cyrenaica, North-eastern Libya: the 'rabbitfishes' (Siganidae) *Siganus luridus* and *S. rivulatus*. The investigated area covers nearly 700 km of Libyan coastlines and two locations in Egypt, and compares traits between and within the two *Siganus* species, from invaded and native areas. The study is expected to advance knowledge of the process of Lessepsian fish migrations and will contribute to the global understanding of successful coastal fish invasions.

The aim of the study can be split into the following three specific objectives (with their related testable hypotheses).

1.3.1. Shape variation in sympatric *S. luridus* and *S. rivulatus*, in relation to their novel and native habitats

a) *S. luridus* and *S. rivulatus* will exhibit interspecific shape differences and intra-specific variance between native and novel habitats.
b) Using complementary data from other components of the thesis, it will be possible to test:
    a. Relationships between morphological and trophic variation.
    b. Relationships between morphological and genetic variation.

1.3.2. Trophic level and niche overlap of *S. luridus* and *S. rivulatus* in native and novel habitats

a) *S. luridus* and *S. rivulatus* will exhibit differences in relative trophic status between native and invaded areas, as a result of changed resource use.
b) Niche overlap/segregation between the two species will be different in native and novel habitats, due to changes in overall ecological interactions.

c) Combining trophic and morphometric data will allow exploration of the potential role of trophic adaptation in affecting body shape.

1.3.3. Comparative phylogeography of the Lessepsian invaders: *S. luridus* and *S. rivulatus*

a) Genetic depletion and founder effect is expected to be stronger in the species that has colonized the Mediterranean for the shorter time.
b) Differences in the degree of spatial substructure can reflect differences in dispersal and connectivity between the two species.
c) Increased genetic diversity can underlie continued influx of Lessepsian migration from areas of origin.

1.4. Study areas and sampling design

1.4.1. Native area of distribution of *Siganus*

Two native populations of *S. luridus* and *S. rivulatus* were studied in the Red Sea. The Red Sea extends between 20.2802° N, 38.5126° E and has a number of unique features. It is the warmest of the world's seas. The climate is equatorial (35-41°C). The average water temperature is 18-21°C in winter and 21-26°C in summer. Surface water temperatures remain relatively constant at 21-25°C. The Red Sea has relatively little water exchange with the Mediterranean Sea and the Indian Ocean, and is regarded as an enclosed Sea. No rivers flow into the Red Sea; this, in addition to high temperatures, results in very high levels of salinity and other dissolved salts. The Red Sea is the most saline sea with salinity of 41 ppm at the Gulf of Suez and 39 ppm of salt at the southern end (the salinity is higher than in the open ocean; Ben Abdallah *et al.,* 2005; Shakman and Kinzelbach, 2007).

1.4.2. Invaded area of *Siganus* along the Libyan coast

The Mediterranean Sea extends between latitudes 30° and 46° N and longitudes 5°50′ W and 36° E. Its west-east extent - from the Strait of Gibraltar between Spain and Morocco to the shores of the Gulf of Iskenderun on the south western coast of Turkey - is approximately 4,000 km, and its average north-south extent, between Croatia's southernmost shores and Libya, is about 800 km. The Mediterranean Sea, including the Sea of Marmara, occupies an area of approximately 2,510,000 km^2.

The highest temperature of the Mediterranean is in the Gulf of Sidra, off the coast of Libya, where the mean temperature in August is about 31°C. This is followed by the Gulf of Iskenderun, with a mean temperature of about 30°C. The lowest surface temperatures are found in the extreme north of the Adriatic, where the mean temperature in February falls to 5°C in the Gulf of Trieste. Ice occasionally forms there in the depth of winter. In the deep zone the temperature range is small, approximately 12.9 °C at 900 metres and 13.1°C at 2,500 metres and temperatures remain constant throughout the year (Ben Abdallah *et al.*, 2005).

The salinity of the Mediterranean is uniformly high throughout the basin. Surface waters average about 38 ppm except in the extreme western parts, and the salinity can approach 40 ppm in the eastern Mediterranean during the summer. Deepwater salinity is 38.4 ppm or slightly less.

Libya presents a coastline of about 2,000 km on the Mediterranean Sea. Its continental shelf covers almost 57,000 km^2, stretching up to 2,000 m deep and most of the area is located in the western part of the country, between Boirat Al-Husnon and the Tunisian border. The Libyan coast has been divided into three main areas of fishing, a western region stretching from Ras Agadir (at the farthest west) to the eastern city of Misurata, then the middle or central region, which ends at the east of Zueitina then Cyrenaica, which ends at the papyrus area, within the region of our study. Libyan shores have abundant natural resources for fisheries that are still untapped in full and have the ability to absorb more investment to increase production after doing a number of environmental and biological studies, which aim to improve the exploitation and management of marine fisheries production (FAO, 1999). Several studies on local ichthyofauna have been conducted on Libyan waters, including a study by Vinciguerra (1881), who recorded 17 species of fish. More detailed studies were conducted during the second half of the twentieth century. For instance, Aldebert and Pichot (1973) studied a variety of flat fishes while Duclerc (1973) concentrated in Scorpaenidae. Additional surveys saw the generation of more detailed checklists, such as one from 1971 which listed 62 species in the western part of the country (Gorgy *et al.*, 1972). In 1977, a total of 131 fish species were registered (Sogreah, 1977). On the same year, 39 cartilaginous fish species were listed, alongside 185 osteichthyes (Contransimex, 1977). Studies by Zupanovic and El-Buni (1982) which made use of demersal fishing gear concluded that Libyan waters had a potentially moderate productivity of fish. The authors further noted that fish fauna in Libya was mainly linked to that found in the eastern part of the Mediterranean Sea, which contains the Levant

Basin. Towards the Benghazi region to the eastern part of Libya, a total of 201 species of bony fishes were listed, which belonged to 71 families and 15 orders (Al-Hassan and El-Silini, 1999). More recent surves (Lamboeuf and Reynolds, 1994; Lamboeuf, 2000). According to Whitehead *et al.* (1984; 1986), the assumption that the entire Mediterranean is composed of similar fish species is unrealistic, based on the observable regional specification in this sea.

1.4.3 Cyrenaica coast

The Cyrenaica province is located in the north-east of Libya, stretching on the Mediterranean coast in the north, to the Green Mountain in the south, and bounded from the east by the Egyptian border and from the west by the Sirte area. Its coastline extends over 1000 km with important portions that are still in very good natural condition. The longest stretch of the Cyrenaicaan coast, with simple coastal structures, dominated by sandy shores, of different sizes and topography, such as the eastern and middle parts represented by flat sandy coasts, with few medium-elevated parts around halophytic vegetations, have freshwater input, giving a brackish-rich habitat. The Cyrenaican coast is mainly rocky and the continental shelf is steep and narrow. The coastal and marine area of Cyrenaica in Libya has been described as one of the "last ten paradises" of the Mediterranean Sea (Bazairi *et al.*, 2013). The coastal area contains sea grass meadows making it an important fish nursery area. Sea turtles *Caretta caretta* nest on the adjacent beaches (Pergent *et al.*, 2012). Two important Mediterranean marine biodiversity hotspots have been identified and protected (Ain algazaland and El-Kouf National Park). Still little information is available from recent year about the biodiversity in the Cyrenaican coast.

The Tobruk coast lies about 130 km from the Egyptian borders. It has a small gulf (about 8 km^2) with commercial, fishing and big petroleum ports. Ain Ghazala is located in a very small gulf (about 60 km). This area has the highest number of small islands in Cyrenaica. Miocene limestone formations cover most of this sector, making coastal slopes of over 40 m east of Tobruk. The remaining coasts vary from low to medium elevated rocky shores to gravel coasts, with limited small sandy beaches, in some areas less than 1000 m long, as in the northern Aïn Ghazala beaches.This area includes alternation of short sandy and sandy/pebble beaches, generally about 1 km long each, intersected by a rocky low to medium elevated coast (Fig. 9). The area is well preserved thanks to limited human access. Together with the nearby Ain Ghazala, it is an important site for sea turtle nesting. The marine waters are shallow with

important seagrass meadows and diverse fish communities. It is an important passage for migratory birds from Europe to Africa.

Figure 9. The sandy beach in the Cyrenaica coast.

The geomorphology of Green Mountain coast (Susah and Al-Haniya) is marked by the presence of coastal slopes and caves, caused by either karstic or marine erosion of the medium elevated limestone coastline. The remaining part is less elevated and specifically rich in coastal lagoons and sebkhas that connect with the sea. The steepest and most elevated Cyrenaican coasts are to be found in this section, as some limestone coastal formations reach >100 m (Susah and Al-Hanyia), with the Green mountain running down to the sea, directly or with a very narrow coastal plain. This area is also relatively richer in coastal headlands (e.g. Ras Buazza, Ras Karsa, Ras Hilal and Ras Amer). The high elevated slopes are intersected with deep narrow wadis, emptying into narrow bays.

The limestone formations of the Green Mountain meet up with a succession of small bays (from 600 m to 4 km wide) including short sandy beaches. The beaches are important marine turtle nesting sites and monk seal have been sighted in the area. Inland, the Green Mountain hills and woodland offers high biodiversity with typical Mediterranean fauna and flora, in near-natural condition.

These areas are characterised by high marine and coastal biodiversity and are in need of immediate action to protect such important hotspots. Three locations in the Cyrenaica coast (Fig. 10), are reported as a well-preserved environment with a variety of diversified assemblages and natural monuments which are unique in the Mediterranean Sea and associated

with an exceptional biological wealth (Pergent *et al.*, 2012). These sites presentsalt marshes, with *Salicornia arabica* and common reed (*Phragmites communis*) and sharp-pointed rush (*Juncus acutus*) fringes and meadoews of seagrasses *Cymodocea nodosa* (which cover 77% of the lagoon) and *Posidonia oceanica.*

The large seagrass meadows at Ain El Ghazala are reported as an indicator of absence of pollution (Reynolds *et al.*, 1995). Pergent *et al.*, (2006) report a list of 26 water birds and 10 terrestrial birds in the area of Ain El Ghazala (Fig. 11). In surveys carried out in 2005 and 2006 (Azafzaf *et al.*, 2006) over 500 birds, including cormorants, waders and gulls, were observed at Temimi salt marsh in both years, while at Ain El Ghazala there were over 300 in 2005 and 600 in 2006, mainly grebes and cormorants, plus some waders, gulls and terns. Ain El Ghazala has been recognized as an Important Bird Area by BirdLife International and has been suggested as a wetland of international importance under the Ramsar Convention and as a Specially Protected Area of Mediterranean Importance (SPAMI) under the Barcelona Convention (Robertson and Essghaier 2001).

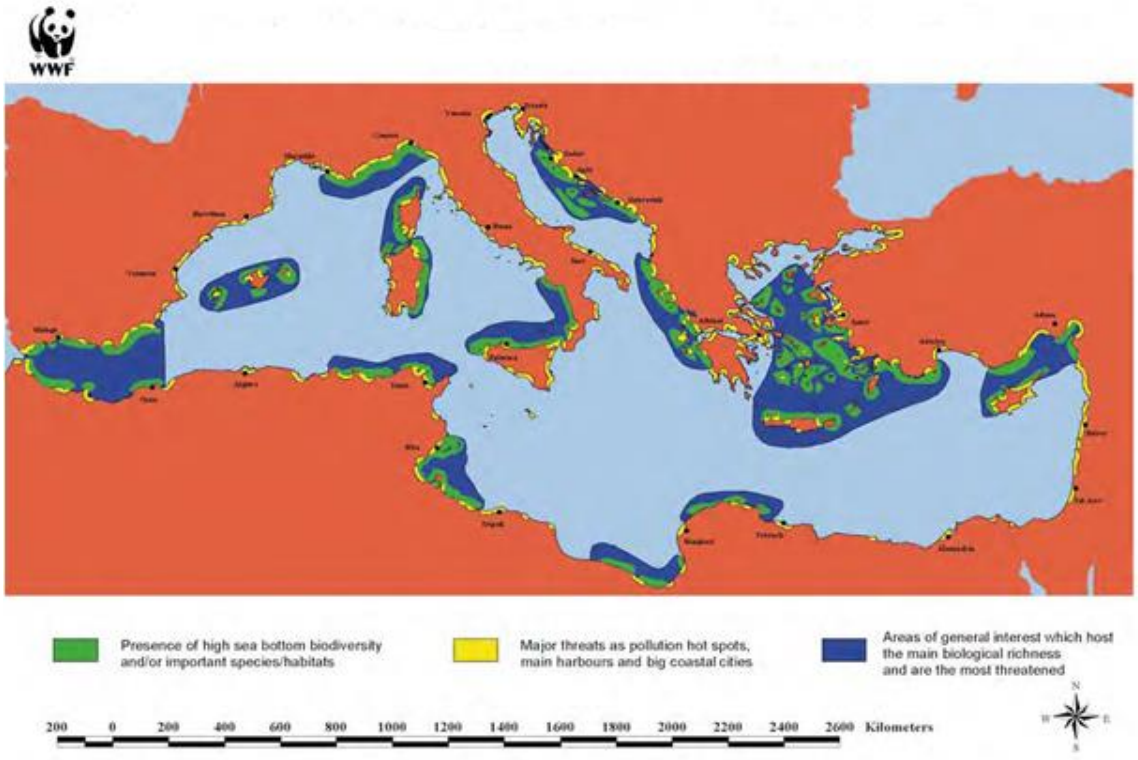


Figure 10. High Mediterranean marine biodiversity hotspots, including three locations in Libya (source: WWF Gap Analysis, 1998).

Figure 11. Vermeted trottoir near Aïn Ghazala. © MF / RAC-SPA1.

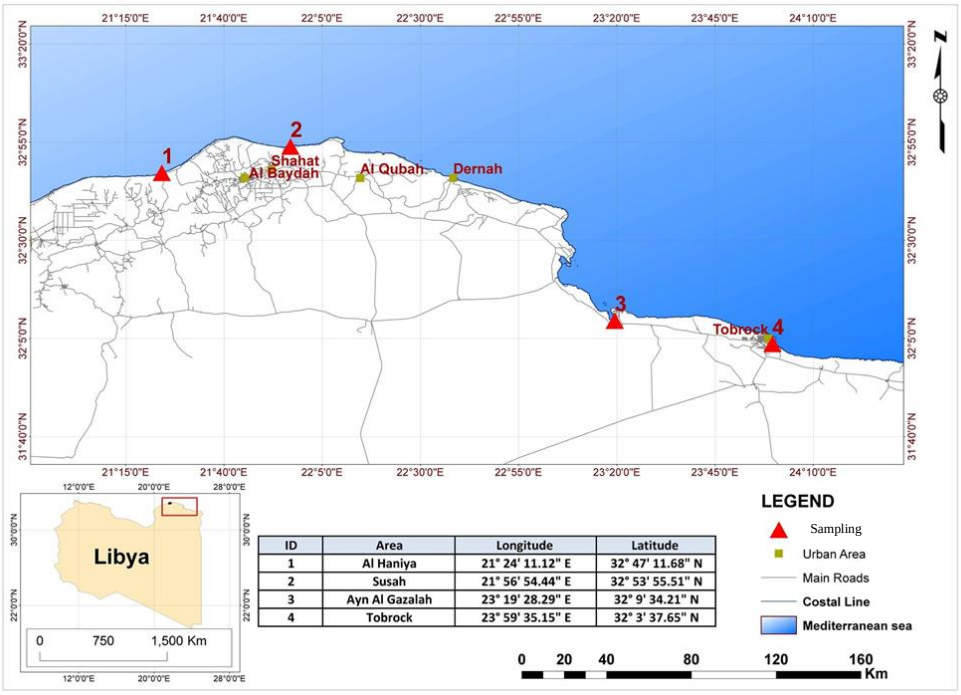
1.4.4. Sampling design

Samples of *S. luridus* and *S. rivulatus* for this study have been gathered from four collection sites in Libya (Fig. 12) starting from the west in Al-Haniya (1), Susah (2), Ain Al-Gazalah (3) and finally Tobrock (4). In the Cyrenaican coast, the marine environment differs from west to east.

The western locations part, Al-Haniy and Soush are more rugged with few sandy beaches and a dominance of cliffs and rocky substrata. The eastern locations part, Ain Al-Gazalah and Tobrock is formed of open and long sandy beaches with sandy bottoms.

In the native area, in the Red Sea, samples have been collected from two areas: El-Tor in the east and Hurghada in the west (Fig.13). Despite the two sampling locations being on opposite coasts, their abiotic parameters are very similar and both have sandy substrate.

Optimal sampling design entails the collection of samples that are representative of the whole or a significant part geographic range. These samples also need to be representative of different seasons in order to allow for an effective investigation of variation patterns.



ID	Area	Longitude	Latitude
1	Al Haniya	21° 24' 11.12" E	32° 47' 11.68" N
2	Susah	21° 56' 54.44" E	32° 53' 55.51" N
3	Ayn Al Gazalah	23° 19' 28.29" E	32° 9' 34.21" N
4	Tobrock	23° 59' 35.15" E	32° 3' 37.65" N

Figure 12. Map of Cyrenaica with sampling locations: 1) Al-Haniya, 2) Susah, 3) Ain Al-Gazalah, 4) Tobrock

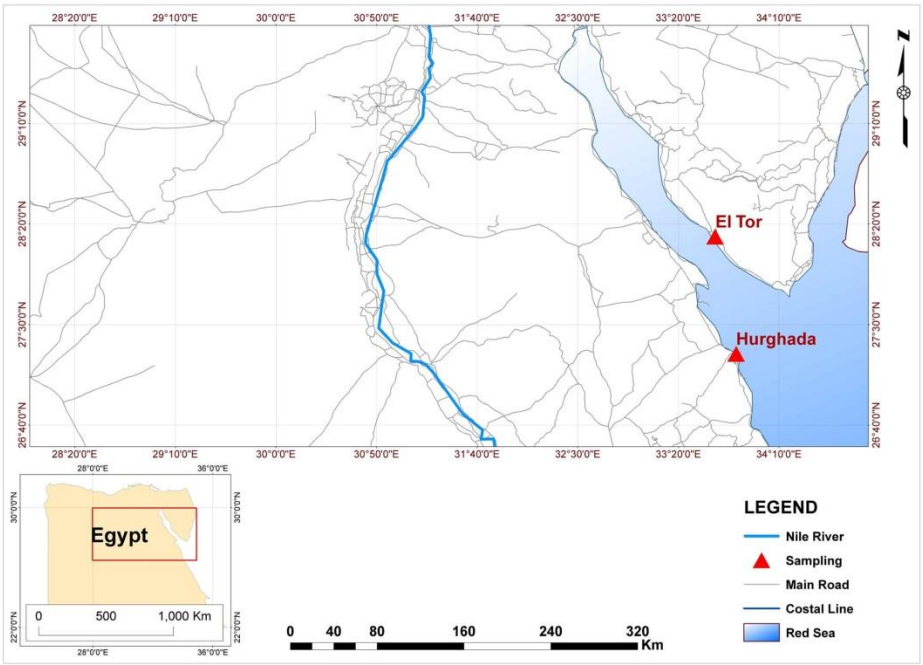


Figure 13. Map of the Red sea with sampling locations: El-Tor and Hurghada

The samples were gathered in October and November 2014 and August and September 2015 directly from local anglers on the coast, as well as fishing boats that fish in the study areas, up to 20 m deep according to the study area and fishing licenses. About 25 samples have

been collected for every species in each area, for a total of 521 specimens (375 specimens from Libya and 146 specimens from the Red Sea; Table 1). According to other studies, a sample size of 25–50 individuals is reasonable to compare populations at the genetic level (Hassan *et al.*, 2003).

Both *Siganus* species show no sexual dimorphism and it is difficult to sex individuals, unless during the reproductive season, when gonads can be analysed (both sampling trips were performed at the end of the reproductive season and only few individuals could be sexed looking at their gonads). In fish in general age is related to size; given the fishing gear all the specimens collected were of similar size (given also legislation for fisheries), so the age of the fish should not vary much in the samples collected.

Table 1. Total specimens collected in the two sampling seasons (October and November 2014; August and September 2015) across six locations in Libya and Egypt.

Location	Country	***S. rivulatus***		***S. luridus***	
		2014	2015	2014	2015
Al-Haniya	Libya	25	25	25	16
Susah	Libya	25	22	25	25
Ain Al-Gazalah	Libya	25	25	20	22
Tobrock	Libya	25	25	22	18
Hurghada	Egypt	22	17	13	15
El-Tor	Egypt	25	19	25	17
		147	133	130	113

Chapter II

Comparison of shape variation in sympatric *Siganus luridus* and *S. rivulatus,* in the Egyptian and Cyrenaican coast using geometric morphometrics

Abstract

Knowledge about body shape is crucial for the understanding of the ecology, life history and evolution of a species, as well as the comparison of populations in different areas. Moreover, patterns of morphometric variations in fishes can reveal observable differences in the growth rates and developmental constraints.The variation in colour is another example of morphological variability within species.

A total of 490 fish were photographed of *S. luridus* and *S. rivulatus* have been gathered from four collection sites in Libya and two collection sites in Egypt in two sampling trips (October and November 2014; August and September 2015).

Image analyses were performed assigning landmarks, using the software MorphoJ v.1.05. Relative Warps Analysis (RWA) was used to analysis of within-population morphometric variation based on landmark data, also Principal Component Analysis (PCA) was used to examine the variation of multiple variables within a single sample, to quantify the colour, and we measured the pixel density for both species using the software ImageJ

The data showed more expanded in *S. rivulatus*; it has more spread out variance between data points, which means there is more variance in body shape. *Siganus luridus* has smaller distribution of the data than *S. rivulatus*, which means the *S. luridus* has less shape variation. The PCA showed slight overlap in morphology between the two species. The colour variations (pixel density) illustrated significant differences in the specimens collected in native habitats, compared to those collected in novel habitats (*S. luridus*: $p = 0.032$, $df = 5$, $F = 645$); *S. rivulatus*: $p = 2e\text{-}16$, $df = 5$, $F = 728$).

The knowledge regarding the biology and ecology of *Siganus* species in the Cyrenaica coast is poor. Certainly, I think it is important not only improving the measurement of traditional morphological variables such as body shape, weight, and length but also providing additional biologically meaningful relationships between these variables and the colour variation.

2.1. Introduction

About 18 Lessepsian fish species have been recorded in Cyrenaica (Sghaier *et al.*, 2013), one of the most important marine environments of the Libyan coast, as it is a favouarble spawning ground for several fish species (Elbaraasi, 2014). Two *Siganus* species, *S. luridus* and *S. rivulatus* have adapted rapidly to the Cyrenaica environment establishing large populations. These fish have become an economically important source for the local community in Cyrenaica.

Fishes differ widely in regards to their body shape and their shape often corresponds to adaptations to various habitats. Sometimes, the causes of morphological differences between fish species are difficult to clarify. It has been proposed that the morphological characteristics of fish are determined by genetic structure, environmental factors and the interaction between them (Muto *et al.*, 2001). Knowledge about body shape is crucial for the understanding of the ecology, life history and evolution of a species, as well as the comparison of populations in different areas (Kitano *et al.*, 2007). Moreover, patterns of morphometric variations in fishes can reveal observable differences in the growth rates and developmental constraints, due to the fact that body form is a result of ontogeny (Azzurro *et al.*, 2014).

Traditional analyses based on dimentions measure the weight of a fish expressed as a function of length (Suresh *et al.*, 2006). Length–weight relationships are a practical index that have been used extensively in fisheries to provide information on the conditions of fish stocks. These parameters are also required to calculate growth rates, age structures and other biological characteristics of fish population dynamics. They are affected by a variety of factors, including diet, in additions to seasons and habitats. Growth is defined as the change in size with time. Furthermore, length-weight relationships are useful for comparing life history and morphological aspects of populations inhabiting different regions. As such, length-weight relationships of invasive species can be valuable for the purpose of comparison between native and novel habitats, providing us with general informations of variation in invasive species growth and specific insights into the ecology of *Siganus* species.

Data obtained from morphometric measurements can also help to identify differences between fish populations. In addition, environmental explanation of morphometric differences could contribute to our understanding of life models followed by different local populations, thus helping to develop a conservation strategy. The word morphometrics simply means measuring form. Many biological disciplines have a need to treat morphological variation in a

rigorous way and geometric morphometrics is considered one of the most powerful methods in the analyses of body shape (Bookstein, 1991; Rohlf and Marcus, 1993). Geometric morphometric methods are being used in a rapidly growing number of studies to examine a wide variety of biological structures ranging from sperm cells to dinosaur skulls (Brusatte *et al.,* 2012). Parallel to numerous other phenotypic approaches, this approach allows for the measurement of a potentially infinite number of morphometric dimensions, in order to facilitate a study of variation patterns. This allows for a study of general morphometry, which entails the shape of the whole organism, or the shape of the various individual features of the organisms.

Due to the manifold possibilities of visualisation geometric morphometric tools are very effective in the presentation of explorative studies. Complex traits such as body shape are hard to study, but with a large set of landmarks it is possible to cover the overall body shape and not only single measurements as in traditional morphometrics. Geometric morphometric thus provides detailed information on often subtle differences in specific body segments or traits. Moreover, the recent development of image processing techniques has improved traditional methods of morphometric identification by facilitating better data collection, more effective descriptions of shape, and new analytical tools (Cadrin and Friedland, 1999). Geometric morphometric methods are also used to quantitatively describe fixed phenotypic differences between closely related sympatric species that segregate in ecological niches (Russo *et al.,* 2008) and may also be employed as 'early detection' methods to investigate hybridization.

Finding the features of shape that can help distinguishing between different groups is an important task of morphometrics in many biological contexts. Distinguishing between groups is not just a matter of looking at the differences between the means of different groups. The variation within groups is also important because it has an influence on the extent of overlap or separation between groups. But in addition to distinguishing between invasive species, we may want to obtain an overview of the structure of intraspecific variation among individuals in native and novel areas.

Morphometric parameters are considered as a key factor in invasion biology because they can explain the effects of the environment on the body shape of invasive species. Traditional length-weight relationship of *S. luridus* and *S. rivulatus* are well studied in large parts of their invaded distribution area, for example in the Mediterranean coast of Egypt, Syria, Turkey, Greece, Italy and Libya (Mouneimné, 1978; Bilecenoglu and Kaya, 2002; Bariche *et al.,* 2009,

2010; Shakman *et al.,* 2008; Shakman and Kinzelbach, 2007). More detailed geometric morphometric studies are missing.

It has been hypothesized that the degree of variation in body shape between *Siganus* species in the Mediterranean Sea and the Red Sea derives from the degree of geographic isolation resulting from immigration (Azzurro *et al.,* 2014). The degree of geographic isolation among various fish species populations has been addressed through a number of population genetic and phylogeographic studies (Spanakis *et al.,* 1989; Bembo *et al.,* 1996; Magoulas *et al.,* 1996, 2006; Kristofferson and Magoulas, 2008). However, information on genetic and phylogeographic population differentiation of *Siganus* species in the Mediterranean Sea is still rather limited (Azzurro *et al.,* 2005; Shakman *et al.,* 2008; Bonhomme *et al.,* 2003 and Hassan *et al.,* 2003).

Variation in colour is another example of morphological variability within species, in addition to the main advantage in the differentiation between the *Siganus* species it's the tail. By extracting colour patterns from picture we can extract quantitative measures of variety in colour that can be used for species comparisons. *Siganus* species possess uniform morphological characters which do not help with identification, thus they are mainly identified based on colour characters. This makes differentiating species difficult, as colour changes throughout their life.

In the present study, *S. luridus* and *S. rivulatus* were sampled in their native area (Egypt) and along the invaded Cyrenaican coast (Libya) to explore length-weight relationships, the variation of their shape using geometric morphometrics and colour change between the native an invaded areas.

2.2. Aims

In this study, basic aspects of image analysis are outlined for *Siganus* species, to account for the difference of colouration within populations in native and novel habitats. Also, the geometric merphometrics analysis was used to compare the shape variation in sympatric *S. luridus* and *S. rivulatus*, in relation to their novel and native habitats, I was interested in understanding whether invasive species changes the colours and body shape in invaded the Cyrenaica coast. Using the software ImageJ and geometric morphometrics it will be possible to test if there is interspecific colours and shape differences and intra-specific variance between native and novel habitats.

2.3. Materials and methods

2.3.1. Fieldwork

Samples of *S. luridus* and *S. rivulatus* have been gathered from four collection sites in Libya (Fig.12) and two collection sites in Egypt (Fig. 13) in two sampling trips (October and November 2014; August and September 2015). About 25 samples have been collected for every species in each area, for a total of 521 specimens (375 specimens from Libya and 146 specimens from the Red Sea; Table.1).

All fish specimens were transported in plastic boxes containing ice to the College of Natural Resources and Environmental Sciences laboratories in Libya and the Faculty of Marine Sciences at the University of the Suez Canal in Egypt.

2.3.2. Length-weight relationships

Each fish was weighted (total weight in g) and weighted again after removing the viscera (eviscerated weight in g). Total length (cm) and standard length (cm) were recorded as well. The data obtained was analysed by fitting length-weight relationships, which can be expressed as

$$W = aL^b \qquad \text{(Hile, 1936; Beckman, 1948)}$$

where W = weight (g), L = length (cm), *a* = rate of change of weight with length (intercept), *b* = weight at unit length (slope). Data were log transformed to estimate the parameters *a* and *b*. When *b* is equal to 3, isometric pattern of growth occurs but when *b* is not equal to 3, allometric pattern of growth occurs, which may be positive if > 3 or negative if < 3.

Length-weight relationship was performed in R (version 3.4.0), by population, location and year. An analysis of covariance (ANCOVA) was performed using weight (log-transformed data) as the response (dependent) variable and “location” as the predictor (independent) variable, using “length” as covariate in PAST for each species.

2.3.3 Geometric morphometrics

A total of 490 fish were photographed from the left lateral side on a white background with a ruler, using a digital camera on a tripod set at the same height, with the same degree of

zoom for geometric morphometric analysis. Samples were labelled according to the study area and the species (Al Haniya (Hn); Susah (Su); Ayn Al-Gazalah (Ain); Tobrock (Tb); El-Tor (Sb); Hurghada (Hu); *S. luridus* (l) and *S. rivulatus* (r) (Fig. 14).

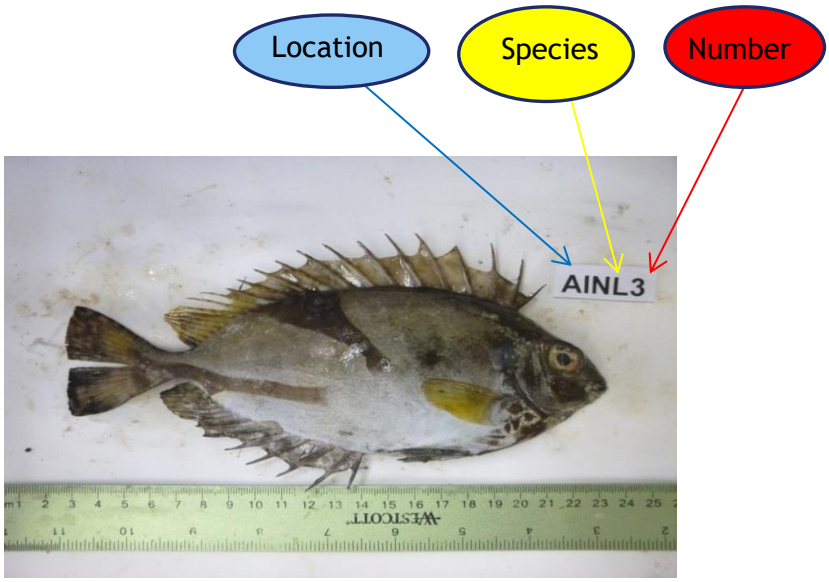


Figure 14. Example of labelling for each individual specimens, including location, species and number

Image analyses were performed assigning landmarks, chosen to define the overall external shape of the fish: 19 landmarks marking homologous points (Fig. 15) were digitised and scaled using the software tpsDIG 2.17 (Rohlf, 2009). tpsSMALL (Rohlf, 2009) was used to ensure the variation in shape among the specimens was not too large for the use of statistical methods utilizing the tangent space approximation (e.g. thin-plate spline methods).

The landmarks were converted to shape coordinates by Procrustes superimposition (Rohlf and Slice, 1990), using the software MorphoJ v.1.05 (Klingenberg, 2011) thus removing information about positioning and orientation from the raw co-ordinates, and standardising each specimen to unit centroid size. Relative Warps Analysis (RWA) was used to analyse within-population morphometric variation based on landmark data. Principal Component Analysis (PCA) was used to examine the variation of multiple variables within a single sample. PCA is often also used for a first exploratory analysis of a larger data set composed of several samples, where it can provide a visual impression of the overall variation. The principal

components are uncorrelated with each other and account for the maximum possible amount of variation.a)

a)

b)

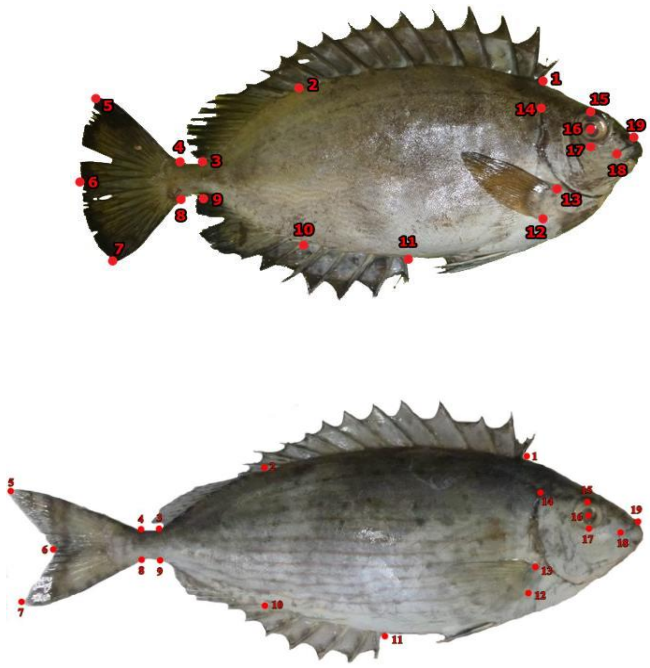


Figure 15. Identification of 19 landmarks used in the geometric morphological analysis for *S. luridus* (a) and *S. rivulatus* (b)

Significance of shape differences was assessed by a Discriminant Function Analysis (DFA) computed on the matrix of partial warps, using MorphoJ. DFA uses a pre-defined grouping of objects and determines to what extent the matrix can efficiently explain this grouping. Canonical variate analysis (CVA) in MorphoJ allows the identification of the shape features that best distinguish between specified groups, and includes a leave-one-out cross validation procedure (1000 permutations) to assess the reliability of classifications. Canonical Variate Analysis functions to differentiate *a priori* defined groups by maximizing the between-group variance relative to the within-group variance.

2.3.4 Colouration measurements

Image analysis methods are quantitative tools for analyzing differences in colours between species, which have been developed to provide quantitative assessment data. In this study, basic aspects of image analysis are outlined for *Siganus* species, to account for the difference of colouration within populations in native and novel habitats.

A basic visual analysis of the *Siganus* specimens revealed variation in colours within species in the different areas. To quatify the colour, we measured the pixel density for both species using the free software ImageJ (https://imagej.nih.gov/ij/plugins/rgb-profiler.html) which is well-suited for analysis of variation in coulors between and within species in native and novel habitats.

Image analyses were performed assigning 26 homologous landmarks using the multi-point tool in ImageJ, to cover the overall external body of the fish (Fig. 16) to quantify the average fish pixel density (the higher the density, the darker the colour).

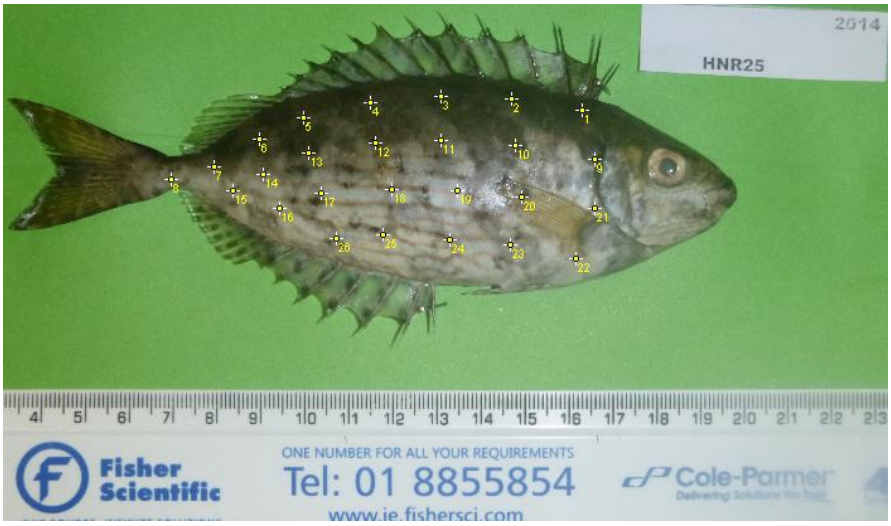


Figure 16. Identification of 25 landmarks used in the ImageJ analysis for *Siganus rivulatus*. The same landmarks were used for *S. luridus.*

To test if colouration of both *Siganus* species differ significantly from different habitats ANOVAs were used. Statistical analysis was carried out using R (version 3.4.0).

2.4. Results

2.4.1. Length-weight relationships

For this study, 490 individuals belonging to two *Siganus* species were sampled. Fish total length ranged between 13.8 cm and 25.6 cm for *S. luridus* (with an average length of 19.75 cm) in the Cyrenaica locations, while, the total length ranged between 12.4 cm and 23.6 cm for *S. luridus* in the Red sea locations (with an average length of 17.8 cm; Fig. 17). The total length ranged between 14 cm and 23.8 cm for *S. rivulatus* (with an average length of 18.9 cm) in the Cyrenaica locations, while, the total length ranged between 15.6 cm and 21.5 cm for *S. rivulatus* in the Red sea locations (with an average length of 18.5 cm; Fig. 18).

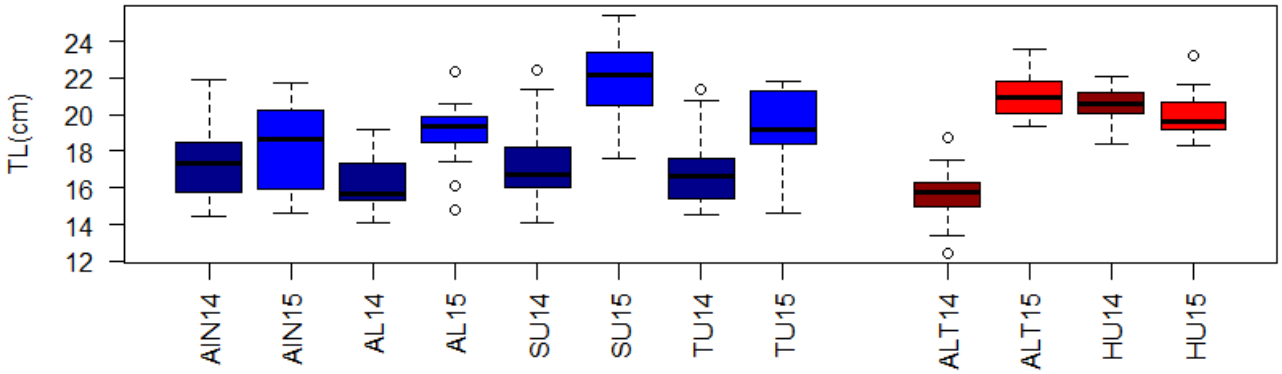


Figure 17. Total length (cm) for *S. luridus* in Cyrenaica (dark blue 2014 and light blue 2015) and Egypt (brown 2014 and red 2015). Individual populations are labelled by location and year (see materials and methods).

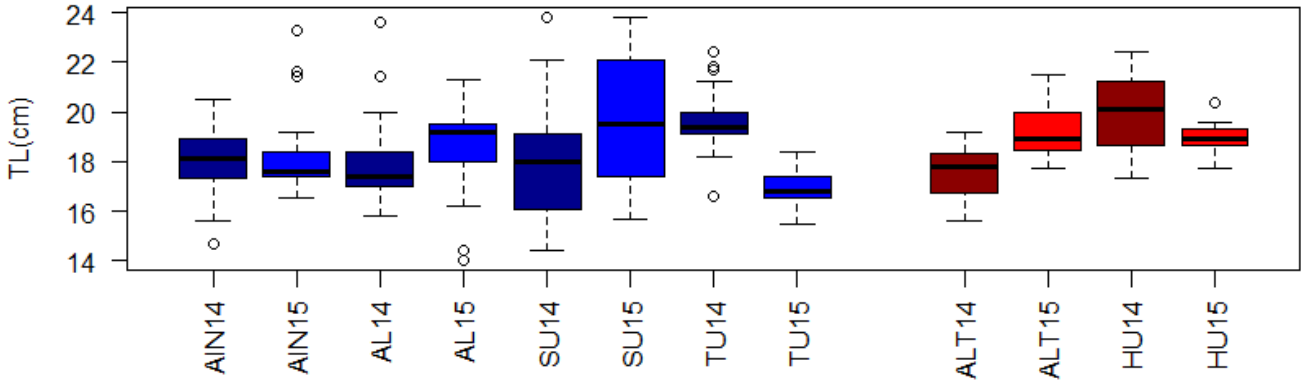


Figure 18. Total length (cm) for *S. rivulatus* in Cyrenaica (blue dark 2014 and blue light 2015) and Egypt (brown 2014 and red 2015).

Individual populations are labelled with location and year (see materials and methods). From the length-weight relationships, values of exponent *b* provide information on fish growth

indicating the type of growth: isometric (b = 3.0), positive allometric (b > 3.0) or negative allometric (b < 3.0). In the present study, the b values ranged between 2.61 to 3.41 for *S. luridus* in the Cyrenaica coast and the Red Sea for the two seasons 2014 and 2015. Our results showed that the type of growth for *S. luridus* in the native habitats and novel habitats were isometric, except in Susah, Cyrenaica, where it was positive allometric (b = 3.34; P = 0.0318; Table. 2). On the other hand, when we compared the two sampling seasons (2014 and 2015) for *S. luridus* the results show that in Susah ($p < 0.001$) and Tubruq ($p = 0.028$) there were significant differences between the two sampling seasons. Also in Hurgadah, in the native area, there were significant differences between the two seasons ($p = 0.028$). The estimated parameters and length characteristics of the length-weight relationship are given in Table 2. For *S. rivulatus*, the b values ranged between 1.80 to 3.19 in the Cyrenaica coast, while the b values ranged between 1.07 to 3.21 in the Red Sea, for the two seasons 2014 and 2015. In the present study we found that the type of growth for *S. rivulatus* in the Cyrenaican coast was isometric in season 2014, except the Al-Haniya was negative allometric in 2014 (b = 1.82; $P < 0.001$). Whole, the type of growth for *S. rivulatus* was negative allometric in season 2015, except the Al-Haniya was isometric in 2015 (b = 3.1; $P < 0.511$). All regression values were found to be highly significant ($P < 0.05$).

Data from all samples were pooled together to describe the relationship between weight -length in the two species in the six regions (Fig. 21 and Fig. 22) respectively. The length-weight relationships for each species in the native and invaded areas are shown in Fig.19 and Fig.20 respectively. When using the length as a covariate factor to compare differences in weight between *S. luridus* and *S. rivulatus* in Cyrenaica coast, it was found that the growth patterns were similar in all the Cyreniaca locations for *S. rivulatus*, also for *S. luridus* the growth patterns was similar in all locations in Cyerniaca except Susah, where was less than all locations in growth patterns. On the other hands, when using the length as a covariate factor to compare differences in weight between *S. luridus* and *S. rivulatus* in the Mediterranean Sea with native areas in the Red sea, it was found that the growth patterns were different among locations in both species. *Siganus luridus* growth was higher in the Red Sea compare to the Mediterranean Sea (Fig. 21; f = 17.57, df = 1, $p < 0.0001$). On the other hand, the growth of *S. rivulatus* was higher in the Cyrenaican coast compare to the Red Sea (Fig. 22; f = 139.3, df = 1, $P < 0.0001$).

Table 2. Total length and for *Siganus rivulatus* and *S. luridus* in the Cyrenaica coast (Libya) and the Red sea (Min: minimum, Max: maximum) and coefficient *a* and *b* from the length-weight relationships $\mathbf{W = aL^b}$ of sampled individuals; $\mathbf{R^2}$ is coefficient of correlation.

Species	Country	Location	Year	n	Min TL	Max TL	*a*	*b*	R^2	P-value	Growth	Difference among year
S. luridus	Libya	AIN AL-GAZALA	2014	22	14.7	21.7	0.0097	3.18	0.859	0.5096	I	0.41
			2015	21	14.4	21.9	0.011324	3.12	0.939	0.505	I	
		AL-HANIYA	2014	25	14.1	19.2	0.038898	2.69	0.876	0.144	I	0.34
			2015	16	14.8	22.3	0.026732	2.83	0.876	0.553	I	
		SUSAH	2014	23	13.8	22.4	0.005897	3.34	0.953	0.0318	A+	6.51E-04
			2015	21	17.6	25.6	0.004866	3.34	0.908	0.127	I	
		TUBRUQ	2014	22	14.5	21.4	0.028359	2.78	0.919	0.244	I	0.0016
			2015	17	14.6	21.8	0.016865	3	0.927	0.98	I	
	Egypt	AL-TURE	2014	25	12.4	18.7	0.023624	2.88	0.941	0.461	I	0.082
			2015	16	19.4	23.6	0.004212	3.43	0.868	0.224	I	
		HURGADAH	2014	12	18.4	22.1	0.005017	3.41	0.783	0.451	I	0.028
			2015	15	18.3	23.2	0.019205	2.94	0.797	0.891	I	
S. rivulatus	Libya	AIN AL-GAZALA	2014	25	16.5	23.3	0.008616	3.19	0.916	0.327	I	0.0004
			2015	22	14.7	20.5	0.056614	2.51	0.929	0.002	A-	
		AL-HANIYA	2014	23	15.6	23.6	0.429772	1.83	0.832	3.92E-07	A-	0.901
			2015	25	14	21.3	0.010581	3.1	0.939	0.511	I	
		SUSAH	2014	22	14.4	23.6	0.012504	3.06	0.795	0.842	I	0.293
			2015	22	15.7	23.8	0.075283	2.43	0.906	0.003	A-	
		TUBRUQ	2014	21	16.6	22.4	0.043963	2.62	0.827	0.1354	I	0.015
			2015	20	15.5	18.4	0.117631	2.25	0.706	0.018	A-	
	Egypt	AL-TURE	2014	22	15.6	19.2	0.006743	3.21	0.864	0.419	I	0.705
			2015	15	17.7	21.5	3.404507	1.07	0.164	0.004	A-	
		HURGADAH	2014	24	17.3	20.4	0.036396	2.64	0.917	0.05	I	0.00022
			2015	14	17.7	20.2	0.06953	2.38	0.244	0.55	I	

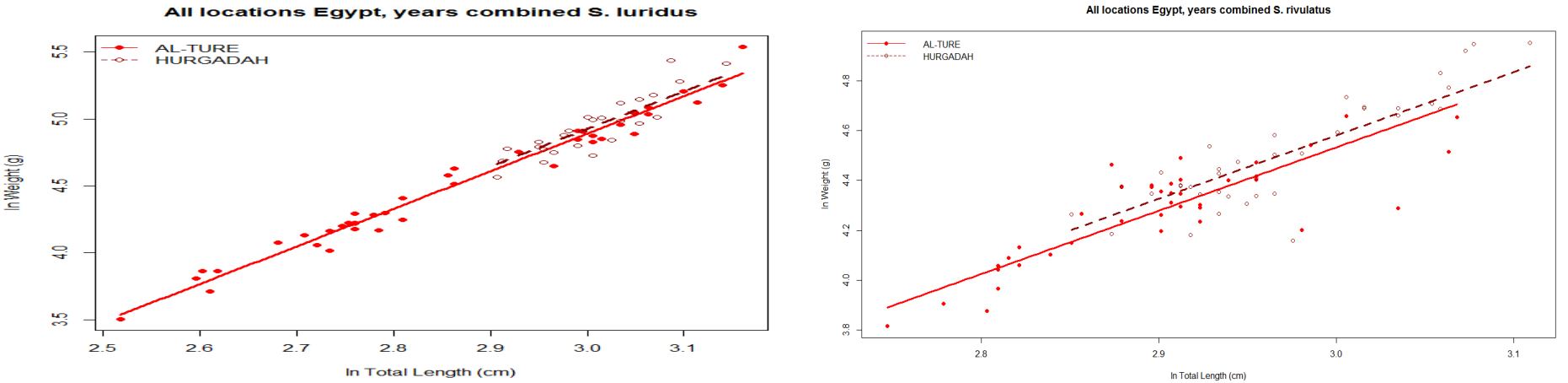


Figure 19. Relationship between length-weight (Log transformed) of *S. luridus* (left) and *S. rivulatus* (right) in Egyptian locations (Al-Ture, Lighr red symbol and Hurgadah dark red symbol).

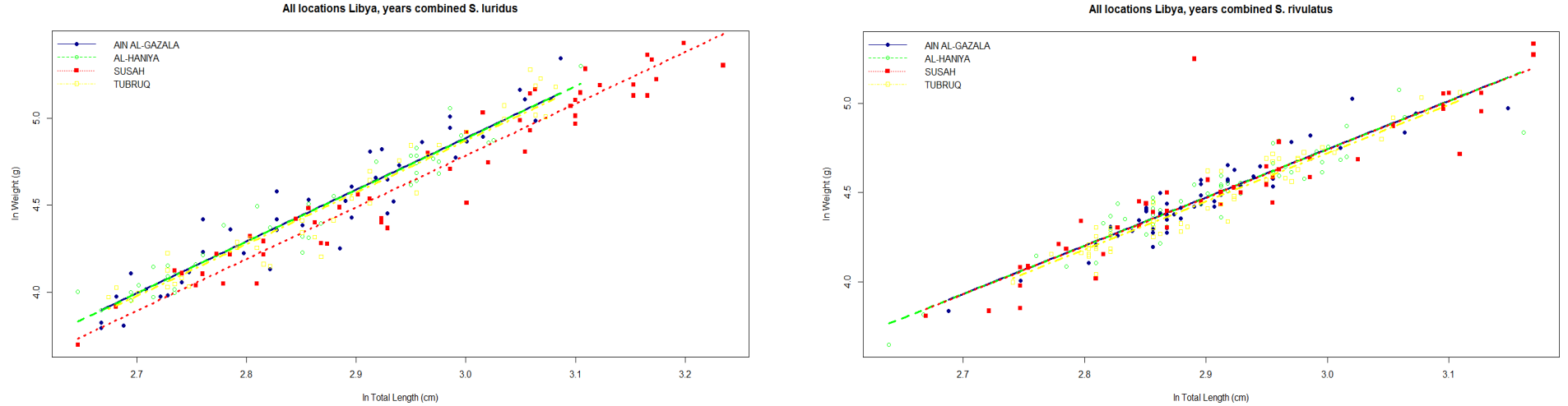


Figure 20. Relationship between length-weight (Log transformed) of *S. luridus* (left) and *S. rivulatus* (right) in Cyrenaica coast (Ain Al-Gazal, Blue symbol Al-Haniya Green symbol, Susah Red symbol and Tubruq Yellow symbol).

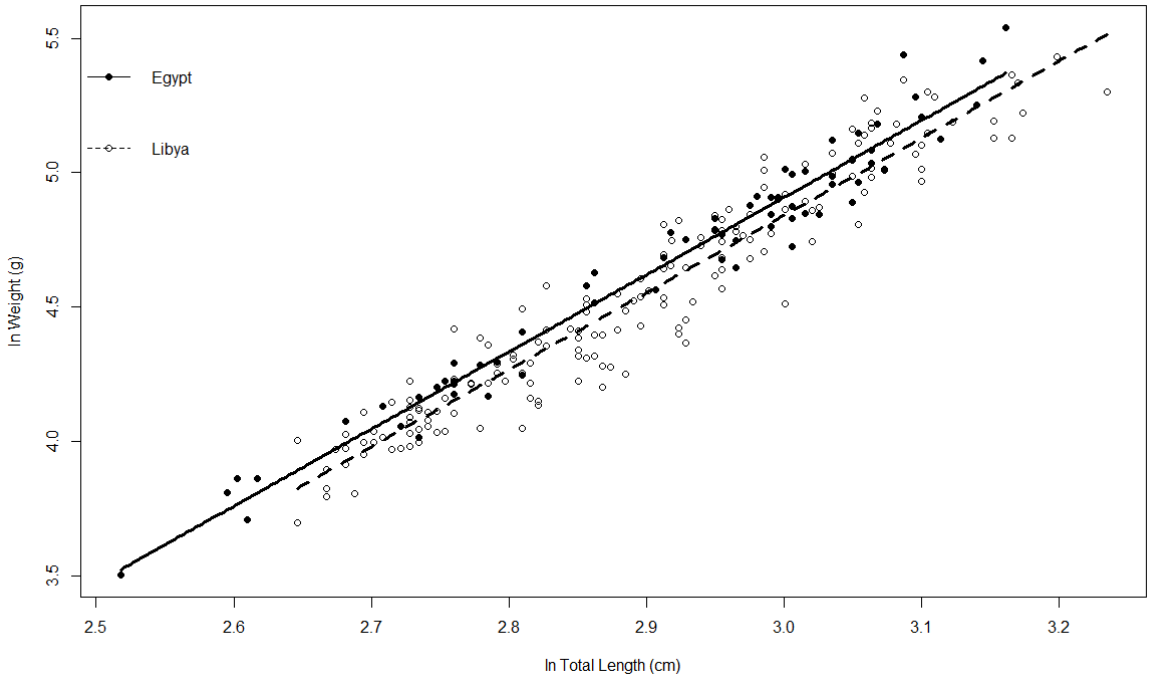


Figure 21. Relationship between length and weight (Log transformed) of *S. luridus* in Egypt (black symbol) and Libya (white symbol).

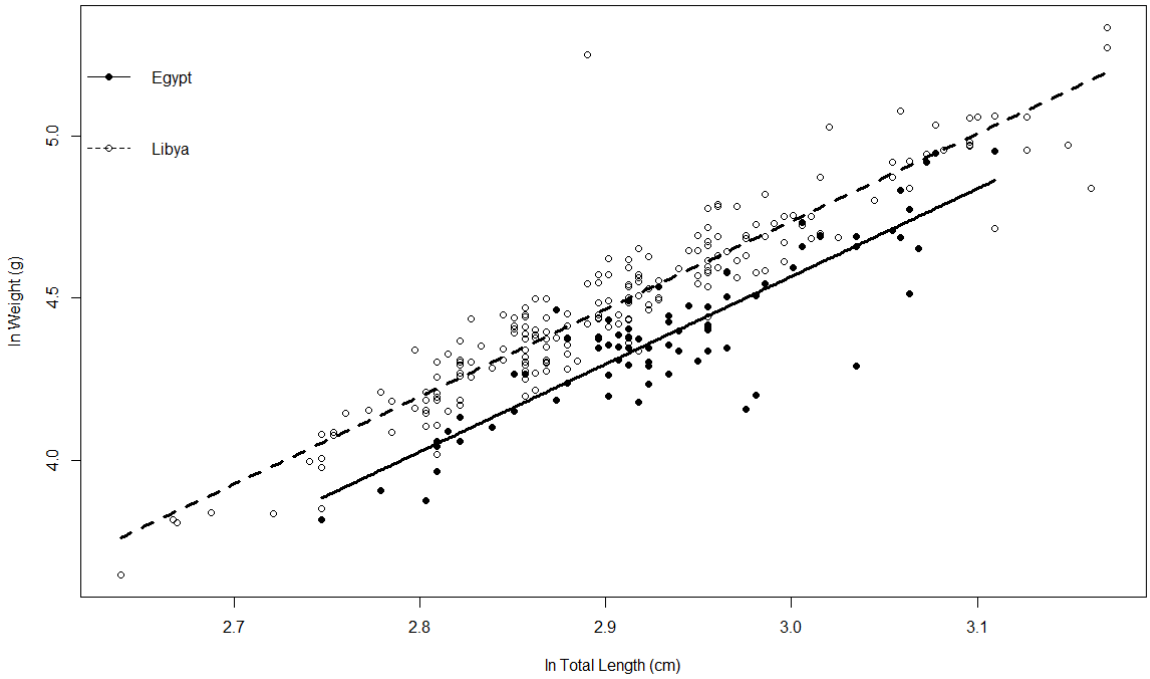


Figure 22. Relationship between length and weight (Log transformed) of *S. rivulatus* in Egypt (black symbol) and Libya (white symbol).

2.4.2. Geometric morphometrics

Using discriminant analysis, we compared body shapes between the two *Siganus* species. The values of the discriminant function have been computed for the 257 *S. luridus* and the 237 *S. rivulatus*. The discriminant function can separate the two groups without any overlap (using 10000 permutations; Fig. 23). The discriminant scores were computed so that threshold for classification to one group or the other is set at a value of zero (Timm 2002). The Canonical Variate Analysis showed differences between native populations in the Egyptain locations than invasive populations in the Cyrenaica coast (Fig. 24). Canonical Variate Analysis of *S. luridus* and *S. rivulatus* in the six study areas shows that the third location in Cyrenaica, Ain Al-gazal (red dots) is mostly separated quite well from the other locations in Cyrenaica and the Red Sea, with the exception of Al-haniya (blue dots).

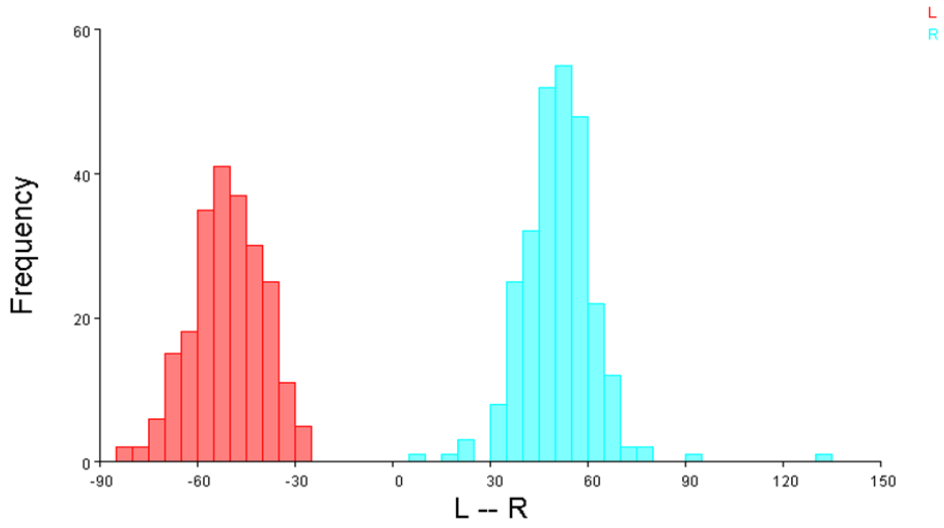


Figure 23. Linear discriminant analysis of the difference in *Siganus* species shape between *S. luridus* (Red) and *S. rivulatus* (Blue) combining the study areas.

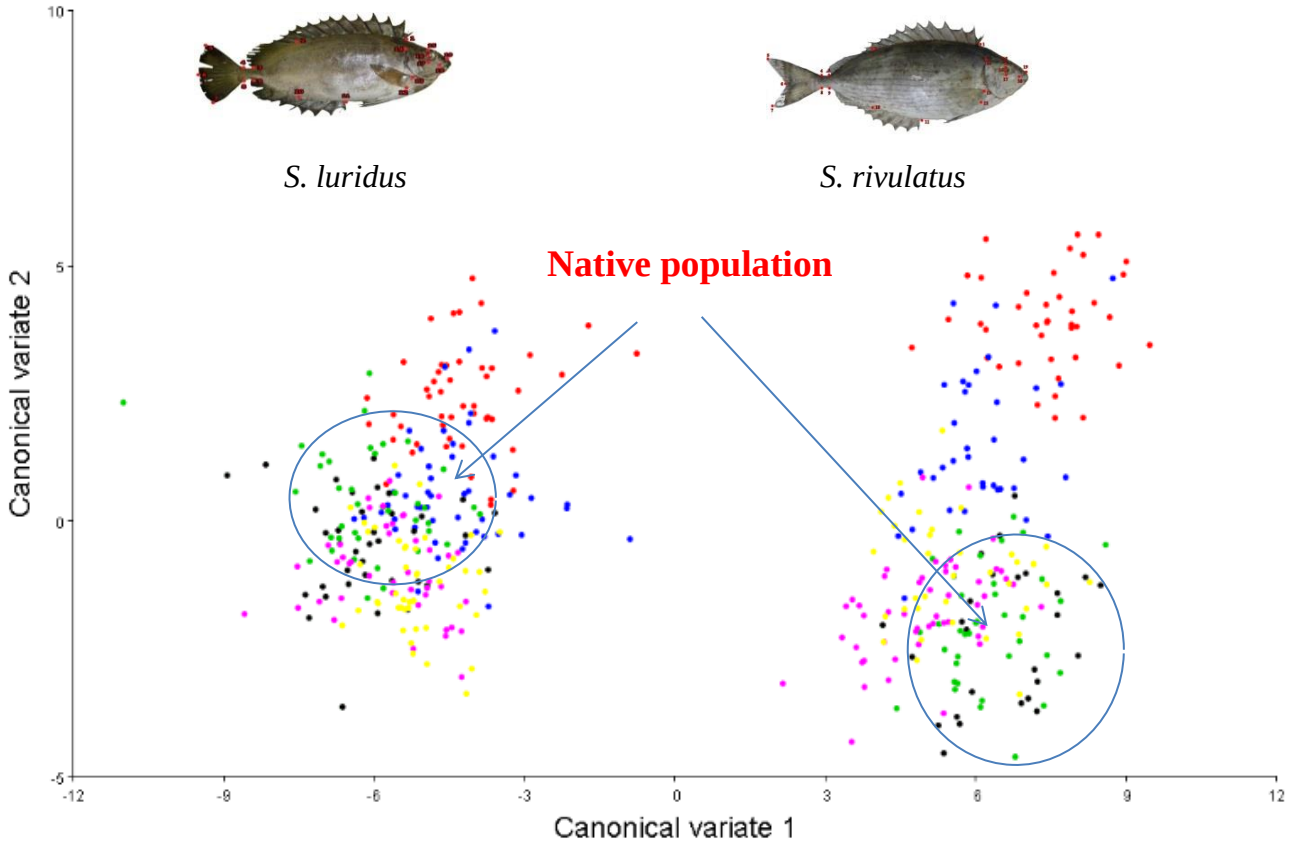


Figure 24. Scatter plot of the first two canonical variates for *S. luridus* and *S. rivulatus*. The groups are defined by species and locations (Al-haniy: blue dots; Susah: Pink dots; Ain Al-gazala: red dots; Tobruq: yellow dots; Hurgadah: Black dots; Al-Tor: green dots.

2.4.2.1. Relative Warp Analysis (RWA)

We looked at some possibilities of visualizing shape changes, mostly using the thin-plate spline. To make shape changes more visible, thin-plate splines were used to draw transformation grids, which show the shape difference from the mean shape in the specimens (Fig. 25). Data provided shown variation in shape between two *Siganus* species in the study areas. This can be observed by the dispersion of the points related to the specimens in the PCA (Fig. 28). The 19 landmarks generated 6 relative warps, one for each location. The grids of hypothetical warps indicate that the specimens obtained from located on a given position in the axes may present warps similar to those of the nearest hypothetical grid. Six groups of *Siganus* species were determined. The groups were obtained from Al-Haniya, Susah, Tubrok and Ain Al-Gazala from Cyrenaica coast and other group aggregated specimens obtained from Hurghadah and Al-Tur from the Red sea (Fig. 26 and 27). That data presented more dilated relative warp grids at the posterior extension of the body shape indicating that the specimens

of *S. luridus* of this groups have body shape relatively wider with grids, indicating that the specimens of *S. rivulatus* present narrower body shape in each study locations (Fig. 25).

In general, the data shown for relative warp grids of *S. rivulatus* indicate that all specimens were the same shape except the samples collected from Susah where, the tail shape is completely different and similar to *S. luridus* (Fig.26). On the other hand, all the samples of the locations have same shape of *S. luridus* but, the samples from Al-Haniya and Ain Al-Gzala were different (Fig. 27), when compared to the samples from the original areas.

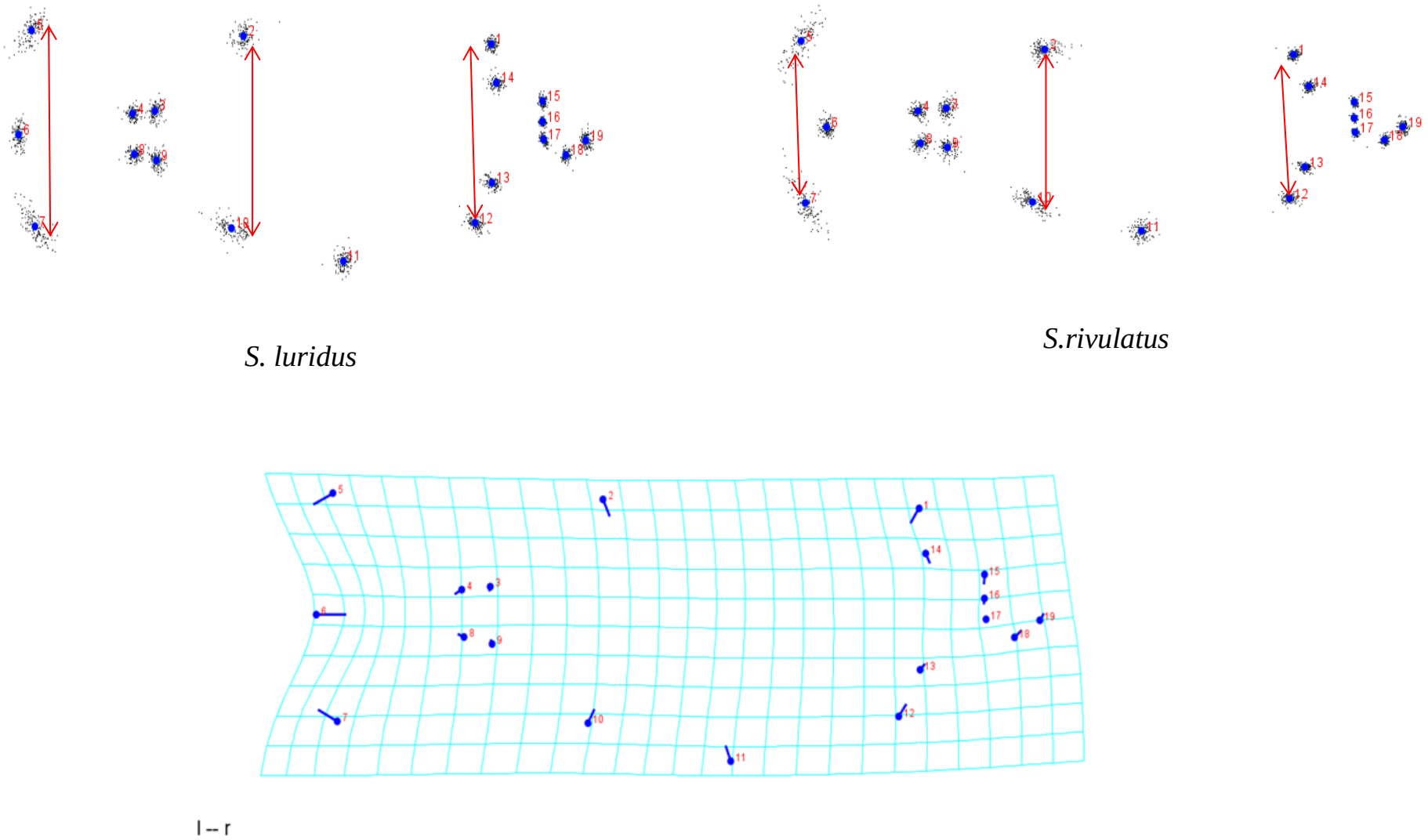


Figure 25. Deformation grids *S. luridus* and *S. rivulatus* obtained with Morphoj and the outline drawings showing the mean shapes for *Siganus* species, visualized by the thin-plate spline

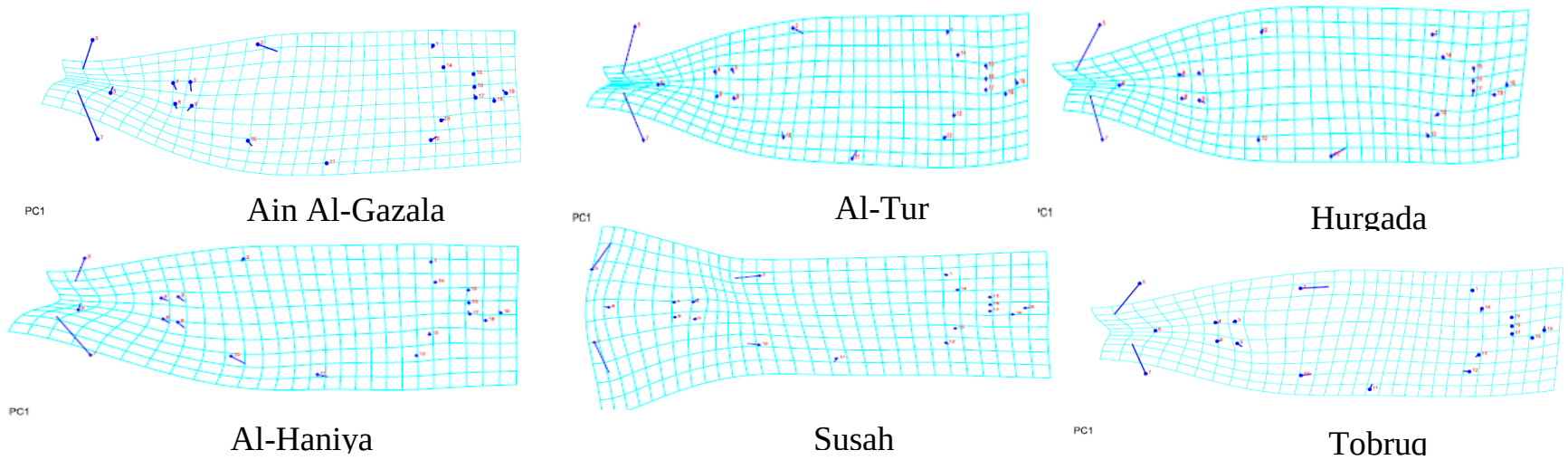


Figure 26. Grids of relative warps with the average shape of *S. rivulatus* specimens obtained from 19 landmarks from study locations

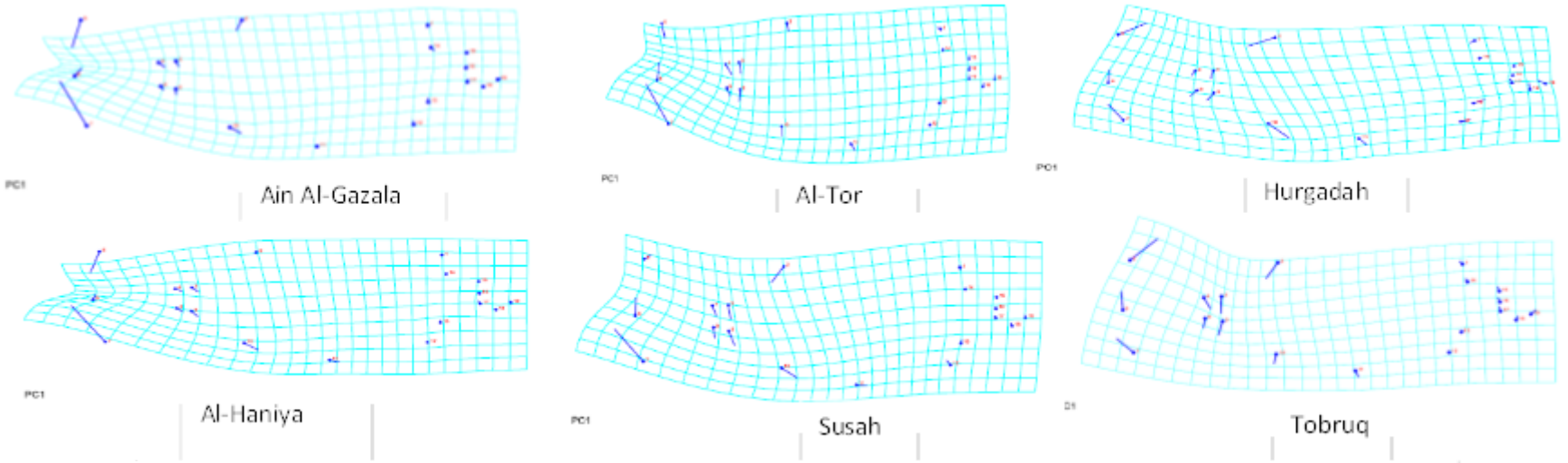


Figure 27. Grids of relative warps with the average shape of *S. luridus* specimens obtained from 19 landmarks from study

2.4.1.2. Principal component analysis (PCA)

Principal component analysis (PCA) is a technique used to emphasize variation between groups and bring out strong patterns in a dataset. It's often used to make data easy to explore and visualize. PCA is generally considered a variable reduction procedure.

The data show (Fig. 28), expressed as a percentage of the total variance, the 30 non-zero eigenvalues decrease from 38% to 0.01%. Among the first two PCs, there is a rapid drop in the variances, but then the values taper off gradually. Accordingly, we can concentrate mostly on the first two. Note that the bulk of the total variance is taken up by about two PCs (Fig. 28). Therefore, the first few PCs are a reasonably good summary of the variation in the whole data set. The variance explained by the first axis is 38.7%, by the second axis is 15.9% and by the third axis is 11.6%, with a cumulative percentage of 66.2% for the three first components.

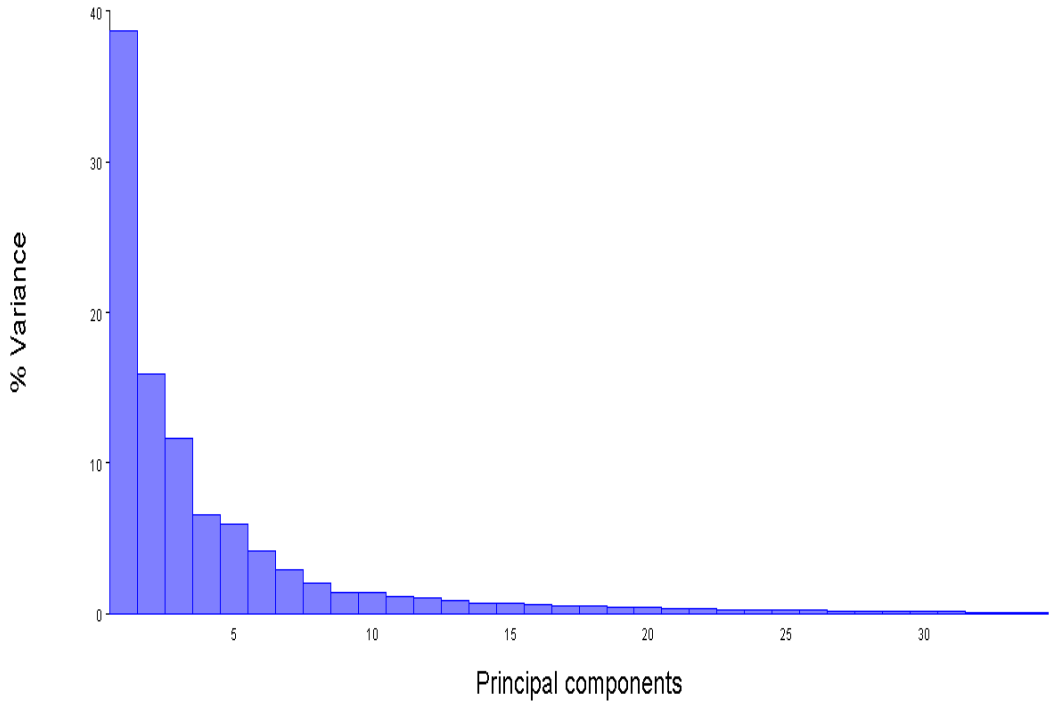
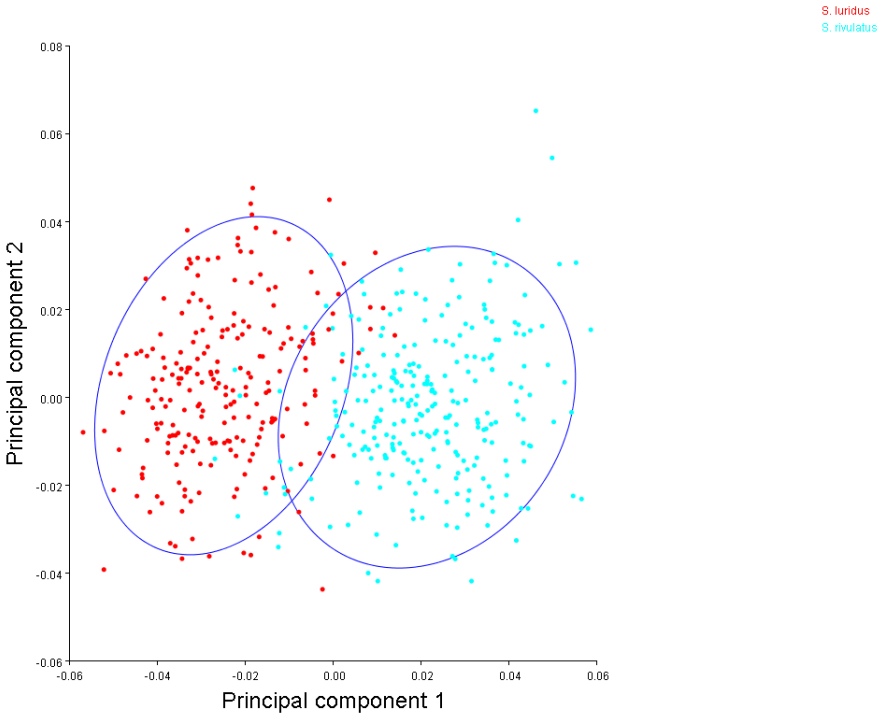
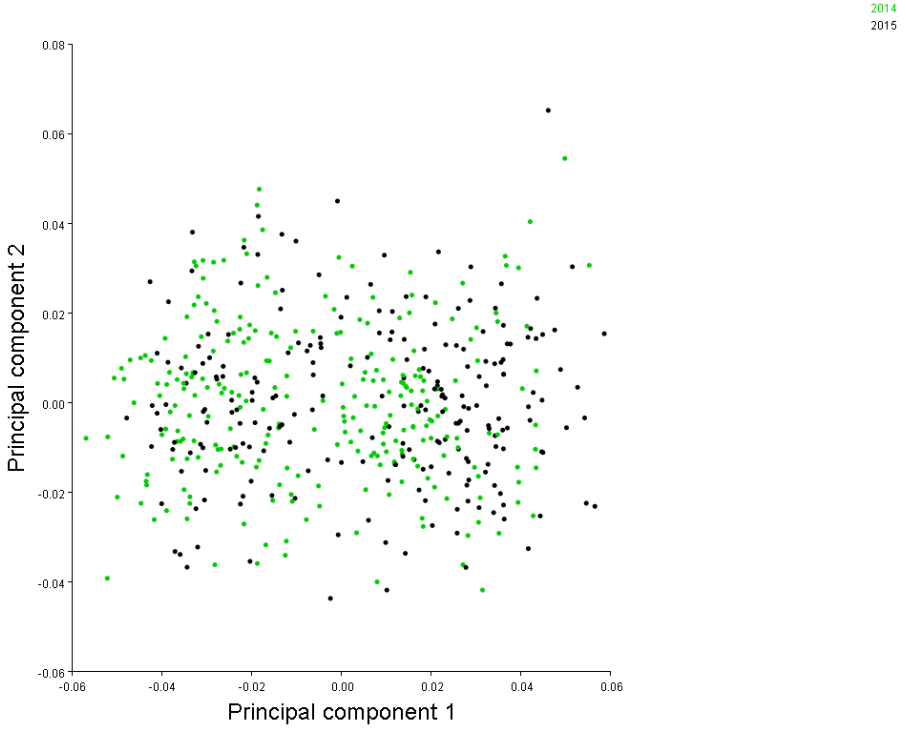


Figure 28. The percentages of total variance for which the PCs account.

The distribution of the data is more expanded in *S. rivulatus*; it has more spread out variance between data points, which means there is more variance in body shape. *Siganus luridus* has a smaller distribution of the data than *S. rivulatus*, which means the *S. luridus* has less shape variation (Fig. 31). The PCA showed slight overlap in morphology between the two species (Fig. 29 and 30). Since many fish species exhibit morphological differences between habitats, the PCA was also used to examine the morphological differences among locations for the two species. This study shows that two species pair follows a different strategy between novel and native habitats. In particular, *S. luridus* and *S. rivulatus* overlap in all study areas coast of Cyrenaica (Fig. 32), while they differ considerably with respect to the body shape in native areas (Red sea) (Fig. 33).

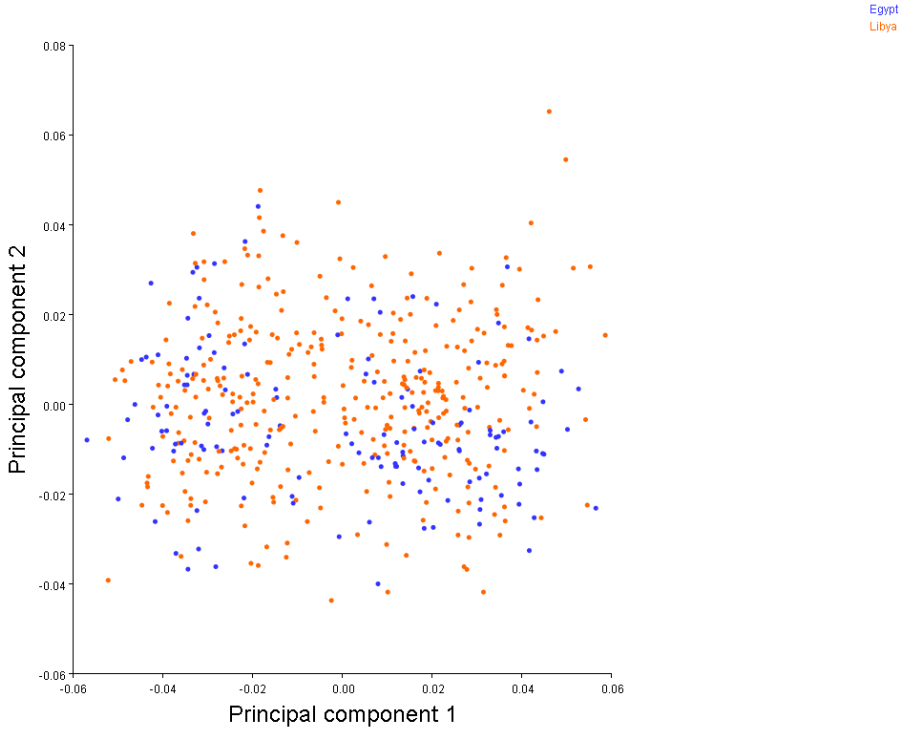


(a). Principal component analyses of morphometric landmarks of *S. luridus* (red dots)and *S. rivulatus* (blue dots)

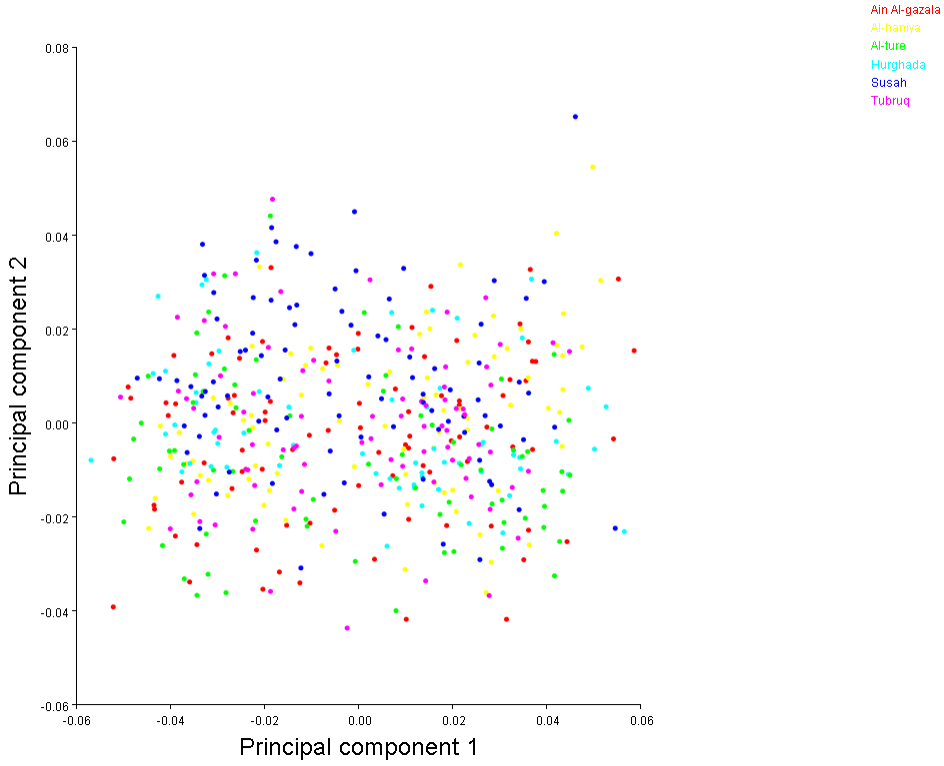


(b). Principal component analyses of morphometric landmarks of sample collection seasons year 2015 (black dots) year 2014 (green dots)

Figure 29. Principal component analyses of morphometric landmarks of *S. luridus* and *S. rivulatus* by (a) species, (b) collection seasons. The PCA showed slight overlap in morphology between the two species regardless of year, geographic area and locations.

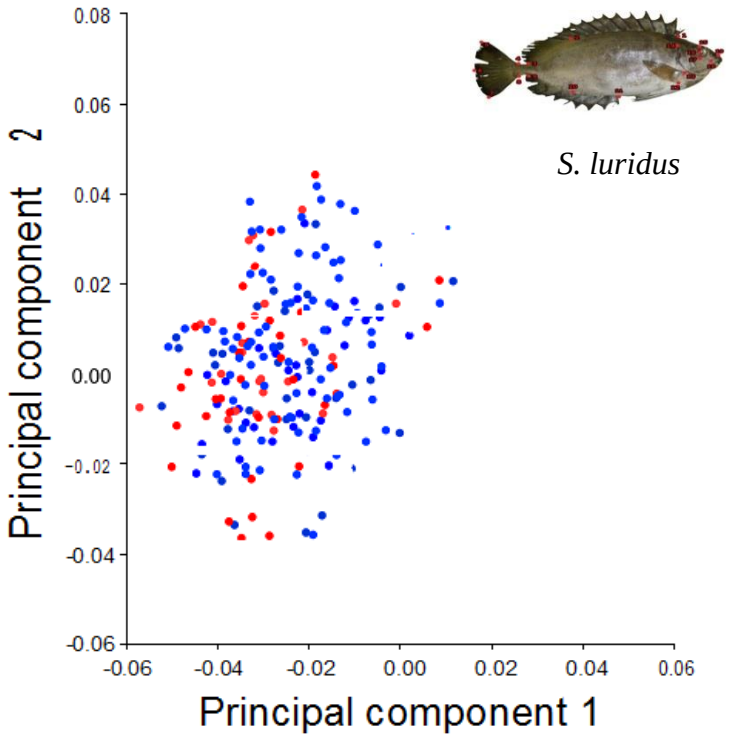


(a). Principal component analyses of morphometric landmarks of S. luridus and S. rivulatus by the country data Egypt (blue dots) and Libya (red dots)

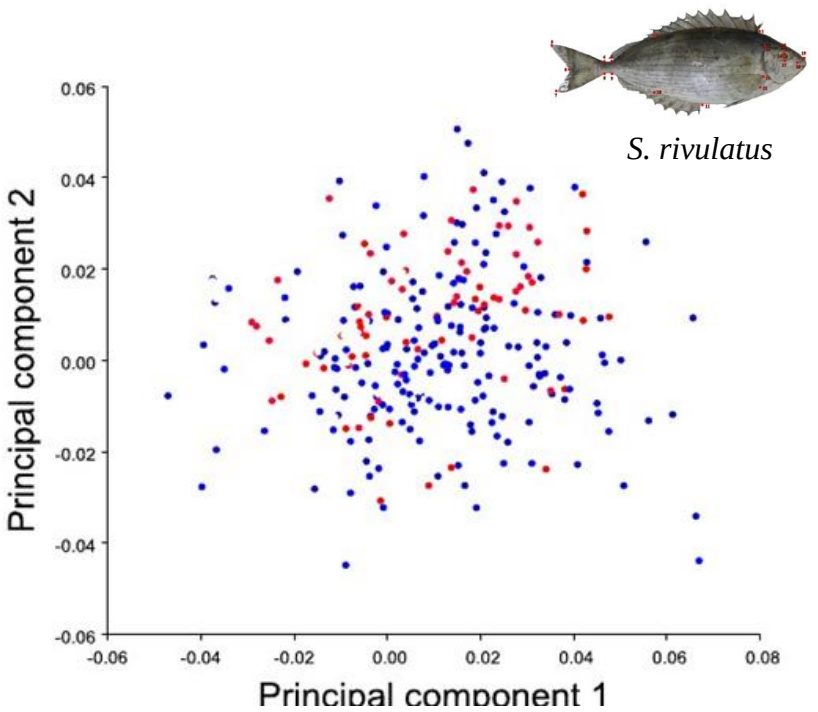


(b). Principal component analyses of morphometric landmarks of *S. luridus*and *S. rivulatus* by locations Ain algazal (red dots), Hurdadah (yellow dots), Al-Tor (green dots), Al-Haniya (light Blue dots), Susah (blue dots) and Tubroq (pink dots)

Figure 30. Principal component analyses of morphometric landmarks of *S. luridus* and *S. rivulatus* by (a) country and (b) locations samples.



(a). Principal component analyses of morphometric landmarks of *S. luridus* by country:Egypt (red dots) and Libya (blue dots)



(b). Principal component analyses of morphometric landmarks of *S. rivulatus* by country: Egypt (red dots) and Libya (blue dots)

Figure 31. Principal component analyses of morphometric landmarks of (a) *S. luridus* and (b) *S. rivulatus* by country: Egypt (red dots) and Libya (blue dots).

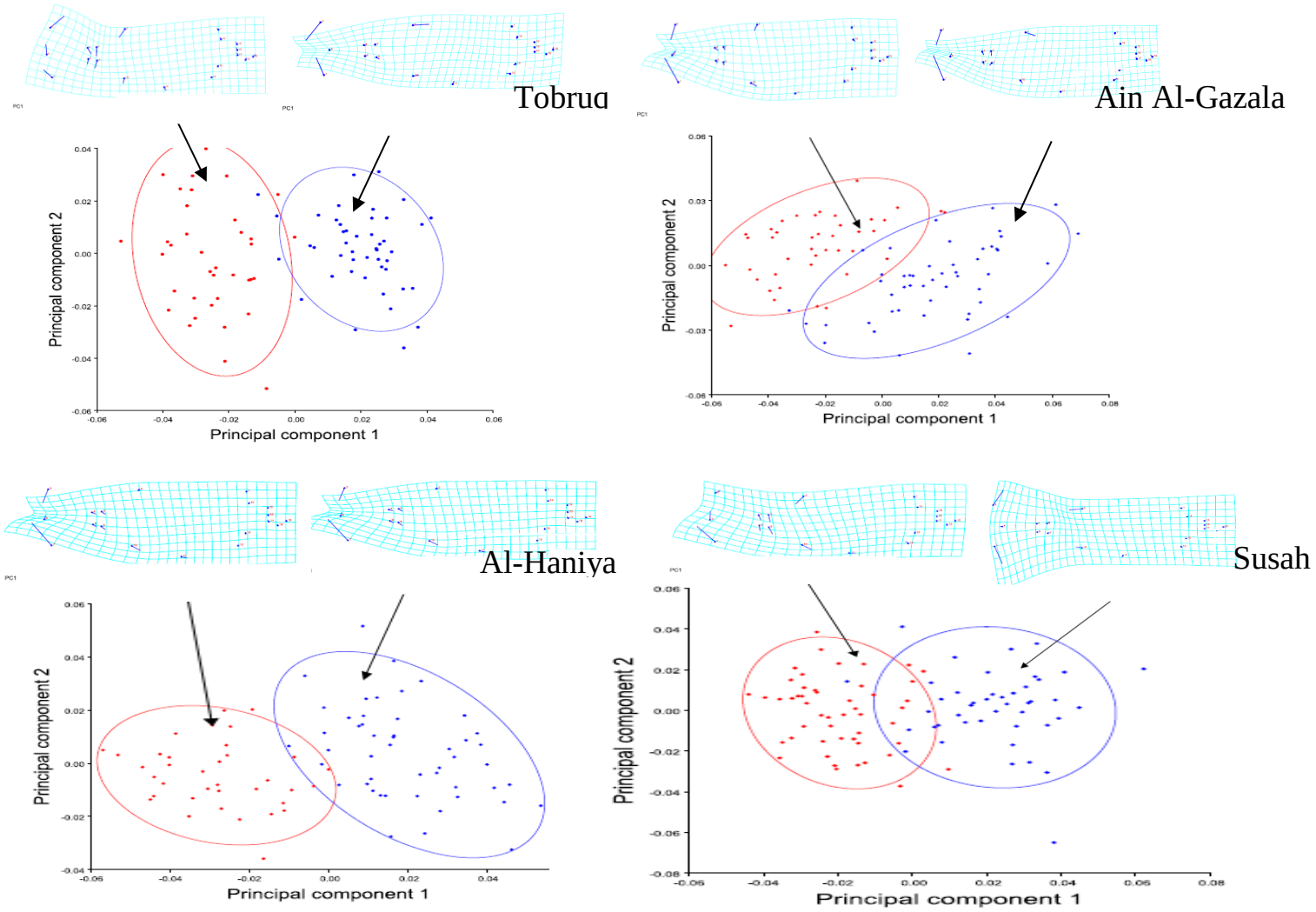


Figure 32. Principal component analyses of morphometric landmarks of *S. luridus* (red dots) and *S. rivulatus* (blue dots) from Mediterranean Sea.Data from two sampling years.

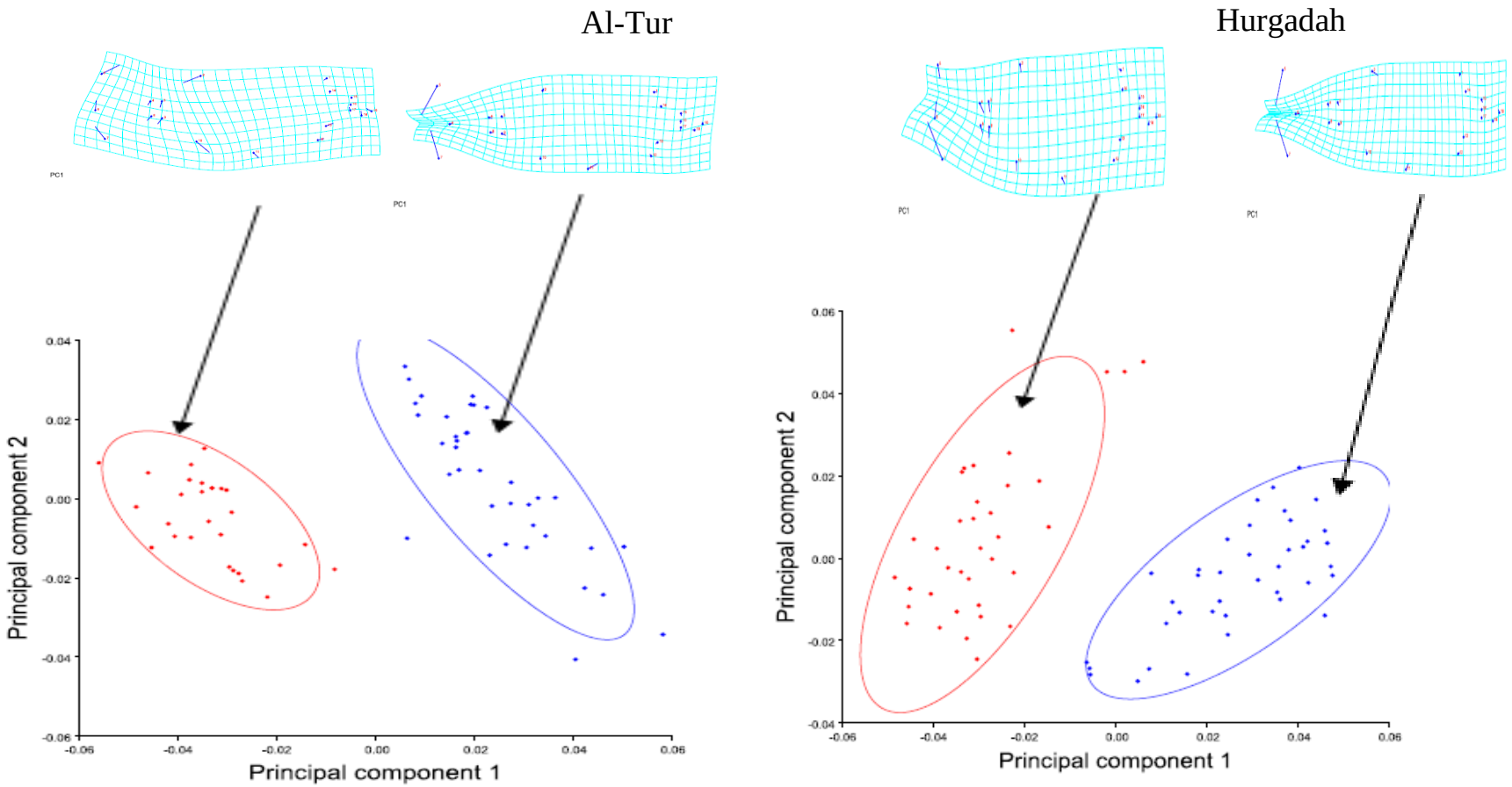


Figure 33. Principal Component analyses of morphometric landmarks of *S. luridus* (red dots) and *S. rivulatus* (blue dots) from Red Sea. Data from two sampling years.

2.4.3. Colouration

Photos of both species from the two areas showed, even just visually that *S. luridus* was almost entirely black (Fig. 34) and *S. rivulatus* white (Fig. 34) in the Red Sea. The same species presented much more variation in the novel habitats (Fig. 35 and Fig. 36). This initial finding was very intriguing, as it could indicate that colours might change in novel habitats in response of different pressures (diverse habitats, food, and even possibly hybridization).

The graph of the measured colour variations (pixel density) illustrated significant differences in the specimens collected in native habitats, compared to those collected in novel habitats (*S. luridus*: $p = 0.032$, $df = 5$, $F = 645$; Fig. 38); *S. rivulatus:* $p = 2e\text{-}16$, $df = 5$, $F = 728$, Fig. 39) and graph from ImageJ revealed that *S. luridus* had darker for colours in Red sea locations than Cyrenaica locations, while, the graph for *S. luridus* revealed that the density values for colours was similar in the Red Sea and Susha in the Cyrenaica coast, though there were differences with the other locations.

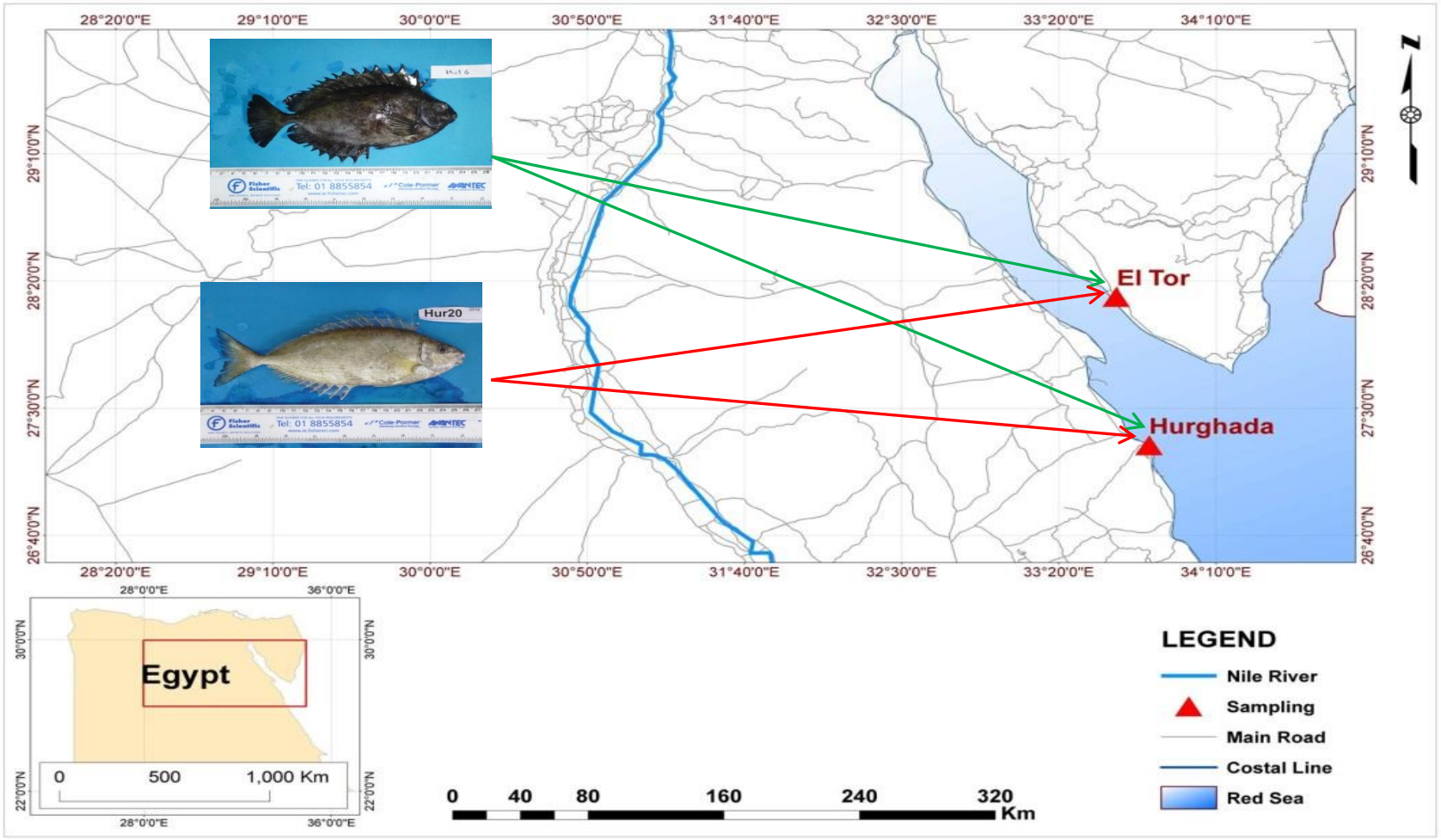
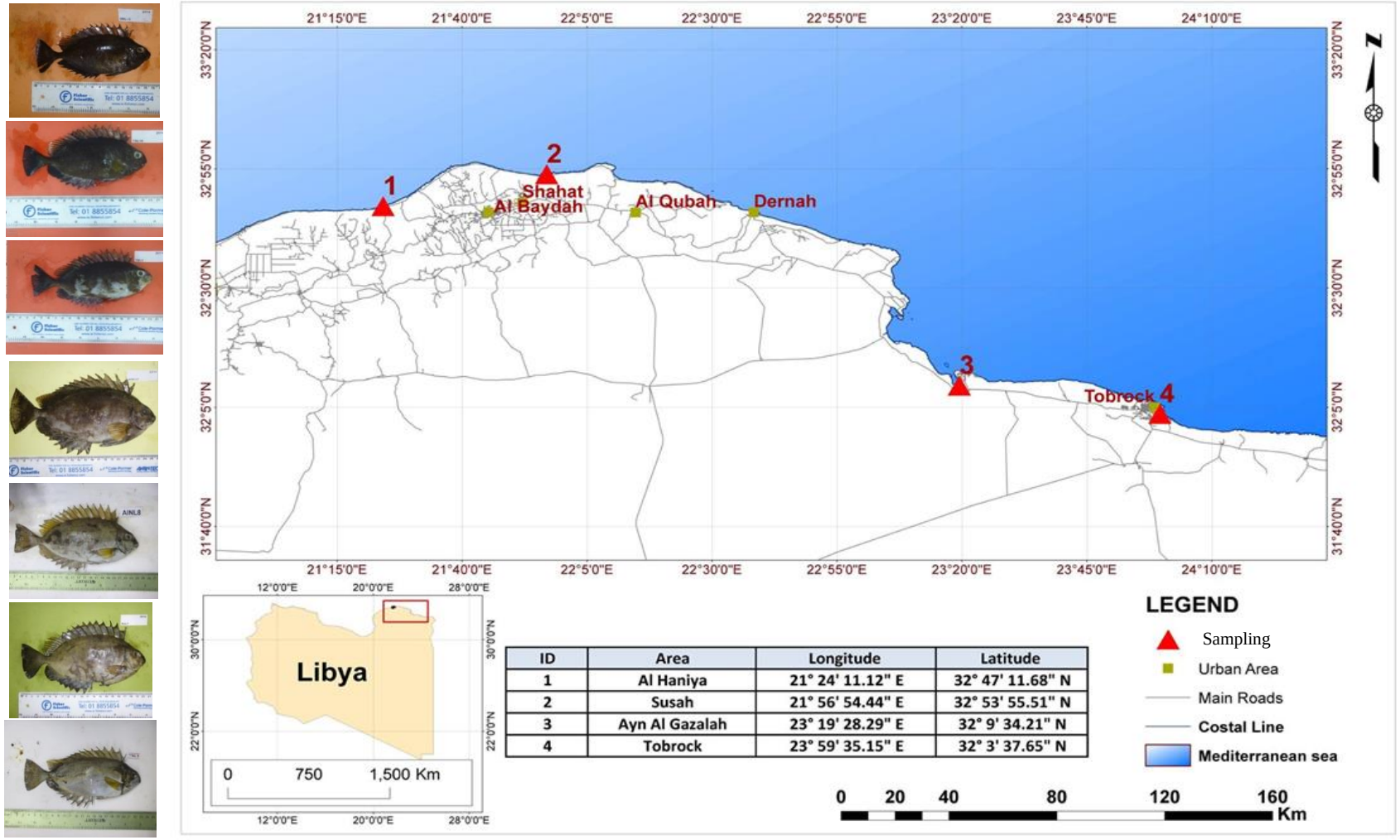
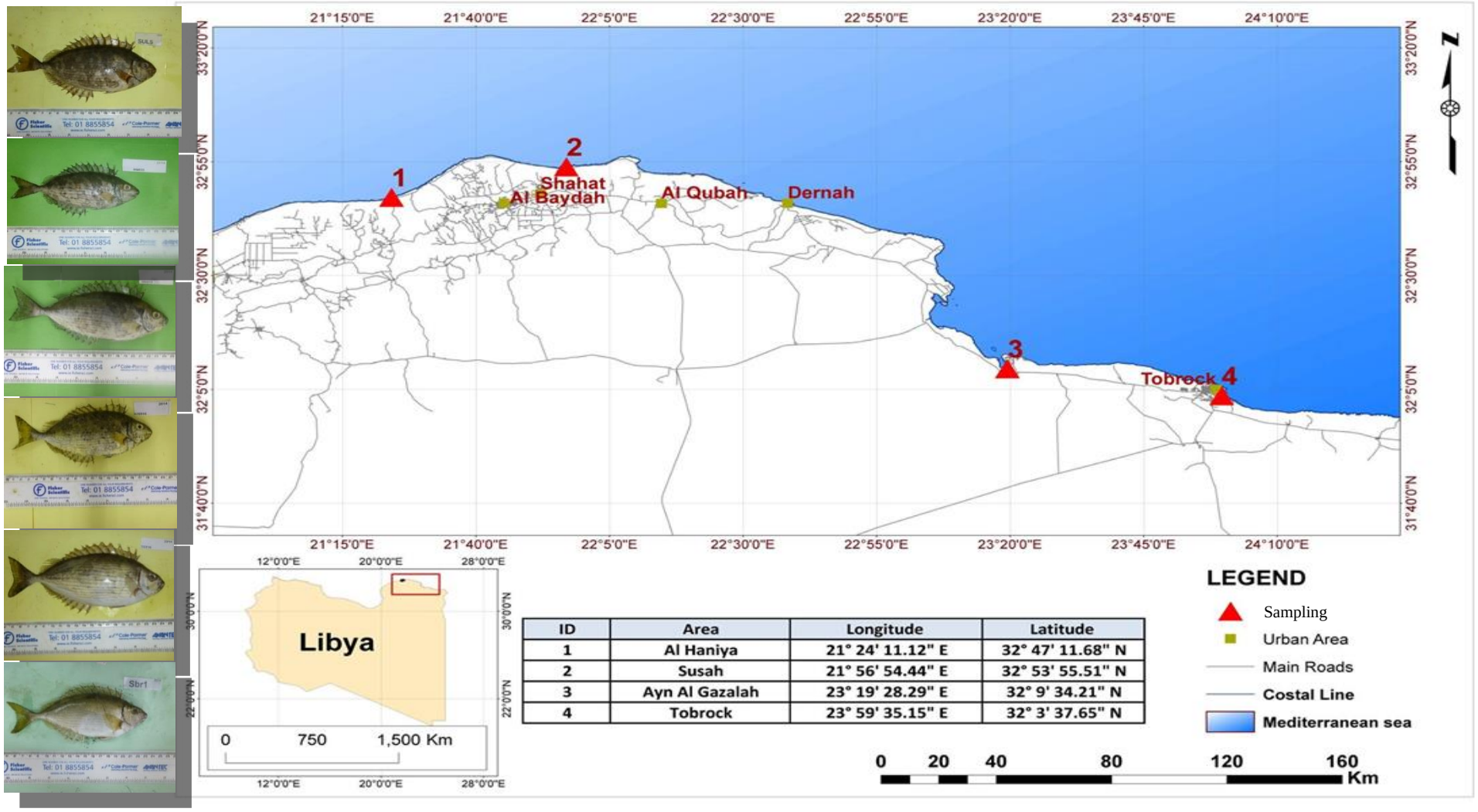


Figure 34. *Siganus luridus* in the Red Sea was almost entirely black, while *S. rivulatus* was white.



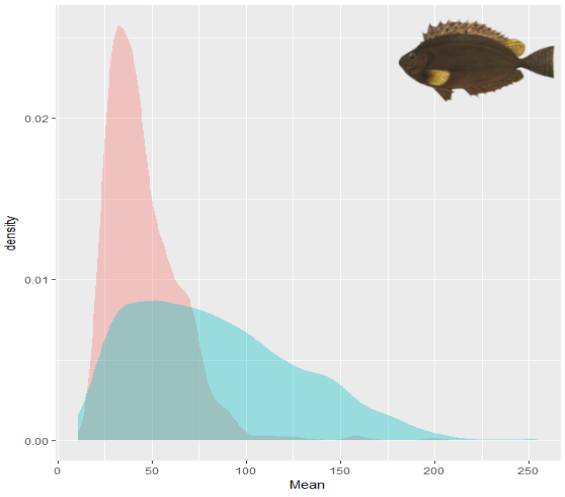
ID	Area	Longitude	Latitude
1	Al Haniya	21° 24' 11.12" E	32° 47' 11.68" N
2	Susah	21° 56' 54.44" E	32° 53' 55.51" N
3	Ayn Al Gazalah	23° 19' 28.29" E	32° 9' 34.21" N
4	Tobrock	23° 59' 35.15" E	32° 3' 37.65" N

Figure 35. *Siganus luridus* in Cyrenaica coast has a grey to black colour gradually, with a light-brown to yellow for dorsal fin.

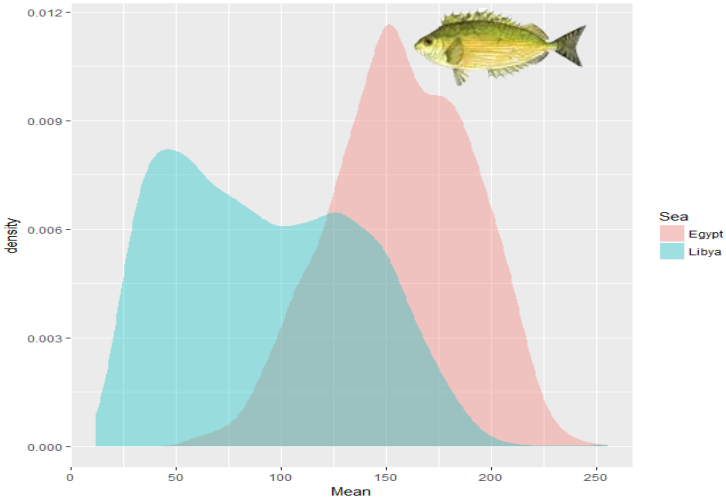


ID	Area	Longitude	Latitude
1	Al Haniya	21° 24' 11.12" E	32° 47' 11.68" N
2	Susah	21° 56' 54.44" E	32° 53' 55.51" N
3	Ayn Al Gazalah	23° 19' 28.29" E	32° 9' 34.21" N
4	Tobrock	23° 59' 35.15" E	32° 3' 37.65" N

Figure 36. *Siganus rivulatus* has two colouration white and splotchy colours.



(a) *S. luridus*



(a) *S. rivulatus*

Figure 37. Colours variation analyses (a) *S. luridus*, and (b) *S. rivulatus*. The colours variation analyses showed differences in colouration between the two species in the two areas (native and invaded).

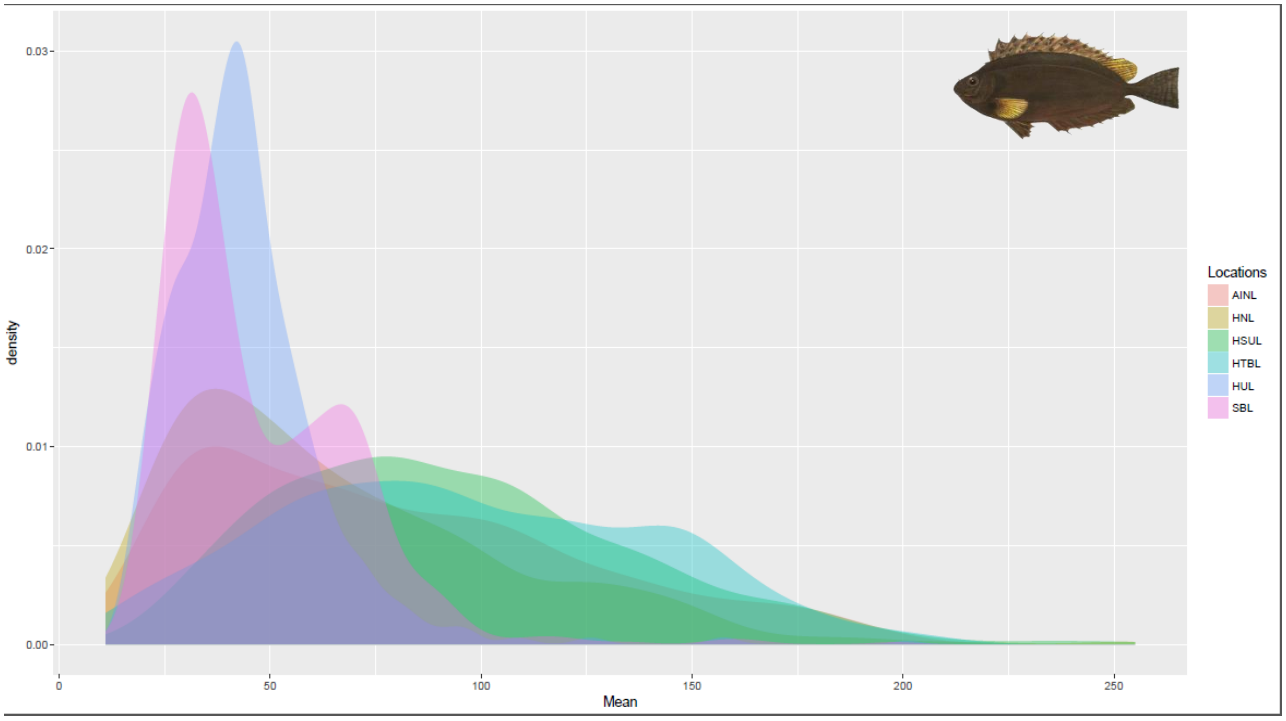


Figure 38. Colours variation analyses of *S. luridus*. The colour variation analyses showed differences in colouration between the two species regardless of geographic area and locations.

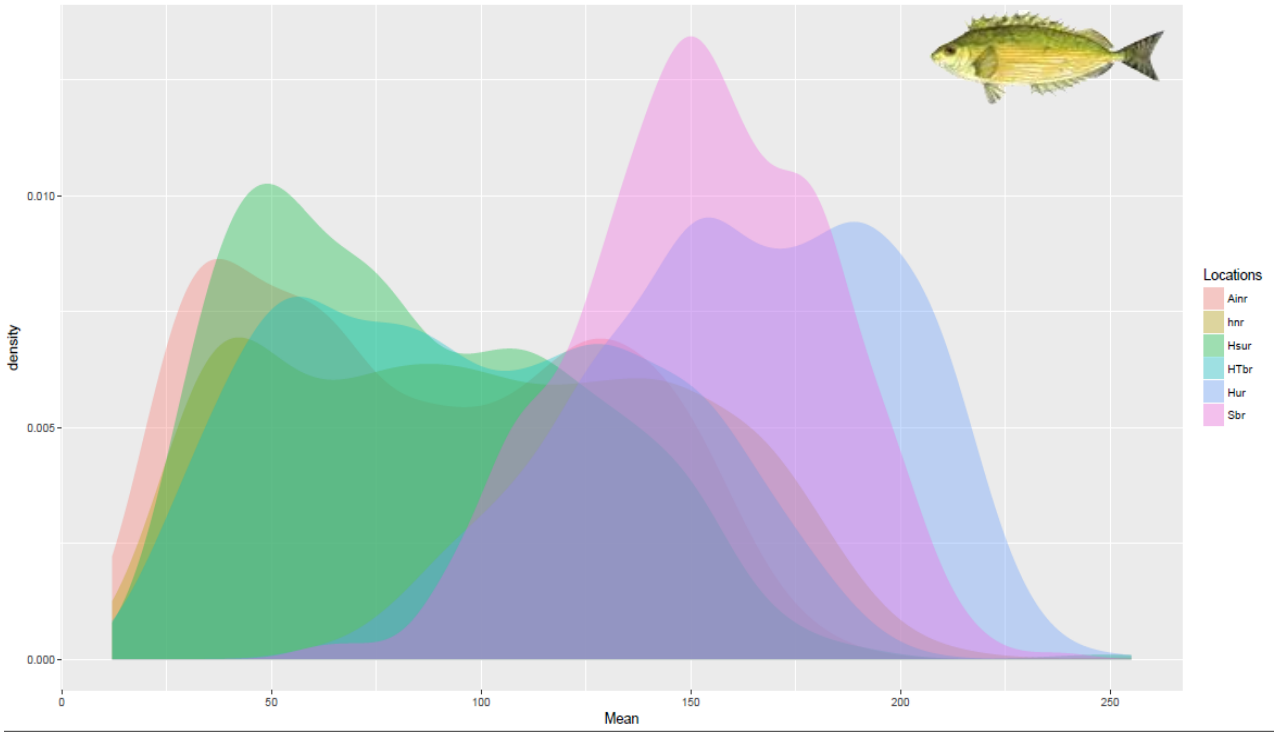


Figure 39. Colours variation analyses of *S. rivulatus*. The colours variation analyses showed different in colouration between the two species regardless of geographic area and locations.

2.5. Discussion

2.5.1. Length-weight relationships

This study presents the estimate of the length-weight relationships for two *Siganus* species of the Cyrenaican coast and compares them with native location in the Egyptian coast. Data showed a slightly isometric growth in all populations. Also, *Siganus luridus* growth was higher in the Red Sea compare to the Mediterranean Sea. On the other hand, the growth of *S. rivulatus* was higher in the Cyrenaican coast compare to the Red Sea.

Similar results were found for the Red Sea population (El-Gammal 1988) and in the south-eastern Mediterranean (Egyptian coast; Abdallah 2002). This difference rates growth is certainly due to different physiological and environmental conditions, which vary with geographical locations (Bariche, 2005). For thetwo*Siganus* species, data were not representative for all months within a year. Thus, these data should be considered as representing only particular seasons or time of year. According to Bagenal and Tesch (1978) and Gonçalves *et al.* (1997), the *b* parameters generally does not vary significantly throughout the year, unlike parameter which may vary seasonally, daily and between habitats. Use of the weight–length relationship should be limited to

the sizes in the estimation of the parameters (Petrakis and Stergiou, 1995; Taskavak and Bilecenoglu, 2001). However, a number of factors are known to influence weight–length relationships in fishes, including growth phase, season effect, size range, general fish condition and size selectivity of the sampling gear (Tesch, 1971).

The reason for different growth rates may be the different environmental factors and the variability of food of these species where the samples were collected (Shakman *et al.*, 2007). Popper and Gundermann (1975) report that the main reason for different growth of populations of the same species in different areas seems to be food habitats, difference of available algae and length of breeding period, which might be different due to temperature differences between the Mediterranean and Red Sea.

2.5.2. Geometric morphometrics

Geometric morphometrics is the study of size and shape of living organisms, where data can be collected in the form of spatial arrangements of landmarks along a biological structure. This powerful technique can capture differences in structures that are not easily observed through traditional types of measurements or by the naked eye. This study applied landmark-based geometric morphometrics to investigate the differences of body shapes in two *Siganus* species. Evolutionary changes in body shape can occur for a variety of reasons, especially for invasive species. The purpose of this study was to investigate the relationship between body shape variation, ecology, and evolution. In many fishes, body shape is closely associated with habitat type. I wanted to answer the following questions: How do *S. luridus* and *S. rivulatus* differ in body shape between the novel and native habitats? How these differences can be quantified? What are some reasonable explanations for these differences? Is the body shape of each *Siganus* species an adaptation to its novel habitat**?**

I focused on body shape differences using geometric morphometrics. Both the discriminant and canonical variate analyses showed that it was possible for the program to correctly defined the two species (both DFA and CVA) with some differences in locations (CVA detected differences in the Libyan location of Ain Al-gazal).

The relative warps detected the differences between *Siganus* species for each one of the different locations. Overall, the shape decomposition of the external morphology showed that the main differences are not spread all over the body but are mainly located in the posterior part of the tail. Also, Susah presented larger tails for *S. rivulatus* compared to all other locations. The PCA showed two separate groups (the two species), regardless of year, geographic area and locations

with some overlap between the two species. The specimens in the overlapping areas will be analysed with genetic tools, to better define to which species they belong.

Results obtained in the present research are not conclusive. However, information from literature indicates that these alterations can occur due to genetic or environmental factors possibly related to trophic ecology.

Fishes present a huge diversity of body shape and the understanding of the importance of their morphology can lend insight into their life history strategies, trace lineages back to speculate on evolutionary processes, and make predictions about how morphological changes coincide with the constant change of their environment. Furthermore, inference about the pace of phenotypic change can be gained from studies involving populations that experienced environmental change at different time periods. Thus, morphological variation is a priceless source of information enriching the knowledge of dynamic processes of ecological systems (Gerber *et al.,* 2008).

In the invasive population of most species, phenotypic variation is determined by genotypic differences between the individuals in native and novel habitats. Moreover, the difference in geographical locations, food availability and environmental conditions affects body shape of organisms. Costa and Cataudella (2007) found that shape differences were related to trophic ecology for several species of the family Sparidae, thus indicating local adaptation (Schluter and McPhail 1992; Langerhans *et al.,* 2003). In order to speculate on the cause of patterns in morphology, experiments should be performed to assess the degree of difference of the trophic ecology within *Siganus* species in Cyrenaica coast and the Red sea locations useing a stable isotope analysis in order to enable the exploration of any connection between trophic ecology patterns and the patterns in morphology (see section on stable isotopes below).

The morphological divergence in shape within invasive species between native and novel locations may be caused by an evolutionary response such as hybridisation (Langerhans *et al.*, 2003). To understand morphological divergence between *Siganus* species in native and novel habitats and explaining morphological divergence, combination of laboratory studies and genetic tests using population markers (e.g., the D-loop and cytochrome *b*) could be used.

The results and knowledge about *S. luridus* and *S. rivulatus* can be used to better understand broader aspects of the biology of body shape evolution in other invasive species in the Mediterranean Sea, to determine if the pattern found in *S. luridus* and *S. rivulatus* can be generalized to other invasive species. The body shape differences are often indicative of adaptation to specific ecological variables. Body shape reflects the processes for immigration and knowledge the change body shape of *Siganus* species provides valuable insight into the macro-evolutionary diversification of major invasive fish groups**.**

2.5.3. Colouration

In the native area, the colourations of the two species are well defined (darker for *S. luridus* and lighter for *S. rivulatus*) while in the invaded area each species presents variations, which might be linked to changes in the habitat. *S. luridus* has been recorded occurring in mainly over rocky habitats in some of the invaded habiatas (Shakman *et al.* 2007), possibly reflecting the original preferred environment, which would result in a darker appearance. On the contrary *S. rivulatus* seems to prefere sandy habitats (Shakman *et al.,* 2007).

Often researchers have underestimated the colour variation at intra-specific level, expecially in invasive species. This study shows associations between the colour variation of *S. luridus* and *S. rivulatus* and various locations in the novel habitat, possibly indicating an expanded use of habitats, a different diet (see chapter 3). One of the patterns of interest we found was the variation in colour range amplitude between *S. luridus* and *S. rivulatus* between native and novel habitats. This result was found for the four locations in novel habitats.

Several hypotheses have been proposed to explain both intra- and interspecific variation of colour. For instance, hybridization and sexual selection also, effect by trophic ecology. Specifically, we focus our predictions on the hypotheses proposed for the change in colours of *S. luridus* and *S. rivulatus* in Cyrenaica habitats: Attack deflection and intimidation of predators. Kelley *et al.,(*2013) famously proposed that the advertisement or 'poster coloration' of tropical fishes might be used to signal aggression in intraspecific territorial disputes, However, the majority of proposed functions relate to predator defence and include camouflage (Kelley *et al.,* 2013). Despite the diversity of colour patterns exhibited by *Siganus* species in the Cyrenaica habitats, the link between, behaviour and colour pattern evolution still remains unresolved. Specifically, there is an absence of studies about the natural enemies for *Siganus* species in the Mediterranean Sea.

It is becoming increasingly clear that the evolutionary histories of various invasive species lineages are affected extensively by hybridisation and its consequences on phenotype evolution. Xu *et al*. (2015) suggest that the development of colour in transparent crucian carp is associated with genetic mechanisms, while Coyne and Orr (2004) suggested that in general in mechanisms of speciation there are two mechanisms of coloration: an initial differentiation involving natural selection for feeding efficiency, followed by more recent differentiation involving sexual selection. However, despite much interest, the underlying genetic mechnisms contributing to their rich variety of colours remain largely unknown.

The other hypotheses: trophic ecology. Zhang *et al.* (2014) pointed out that, striped body patterns in butterflyfishes showed correlation with a number of ecological factors including habitat type, sociality and dietary complexity. On the other hand, Zhang *et al.* (2014) suggested that the spots and eyespots in butterflyfishes are unlikely to have played an important part in the evolutionary history of the butterflyfish. Seehausen *et al*. (1999) pointed out that the stripes have different functions; in cichlid fishes where, the horizontal stripes are associated with social behaviour and foraging mode while vertical stripes are linked with habitat type.

The "trophic ecology" hypothesis proposes that evolution of interspecific variation in body colour is driven by variation in trophic ecology environments across habitats. If ambient nutrition has the potential to drive interspecific variation in the body shape, as colour signals are an adaptive response to the change in ambient nutrition levels in different habitats.

A major challenge for future work is to establish how genetic analyses and trophic ecology are related to colour change of *Siganus* species in the Mediterranean Sea. Answering these questions will be important for evaluating the relative importance of extrinsic ecological conditions and geographic distribution of *Siganus* species' colour variation. The research of *Siganus* species colours variation will become more integrative. The genomics revolution allows both deeper and broader understanding of the mechanisms underlying colour variation, and these insights will be particularly informative when accompanied by ecological and behavioural studies. In this way, we can take full advantage of the *Siganus* species model system as a tool for understanding the evolution of biological diversity for the in vasive species in the Mediterranean Sea.

Regrettably, as mentioned above, the knowledge regarding the biology and ecology of *Siganus* species in the Cyrenaica coast is poor. Certainly, I think it is important not only to improve the measurement of traditional morphological variables such as body shape, weight, and length but also providing additional biologically meaningful relationships between these variables and colour variation. The knowledge and understanding of colour variation in invasive species will allow researchers to identify the environmental influences on colour variation and to evaluate the observed differences in an appropriate frame of interpretation.

Chapter III

Trophic level and niche overlap of *S. luridus* and *S. rivulatus* in native and novel habitats

Abstract

The Mediterranean Sea is the most invaded marine region of the world, providing us with a unique unplanned experiment to understand how biological invasions are affecting food web properties in the Mediterranean ecosystems. Invasive species can modify trophic ecology structure and change ecosystem-level functioning, as invasions create new links altering interspecific relationships at the different trophic levels in the new habitats.

In this study, stable isotope ratios $\delta^{15}N$ and $\delta^{13}C$ were used to quantify trophic relationships for *S. luridus* and *S. rivulatus* in the Cyrenaica coast can allow comparisons between trophic patterns between the Mediterranean and the Red Seas, as well as comparing to its native habitats in Red sea. A total of 394 specimens belonging to two *Siganus* species and 9 specimens for algae and sea grasses.

Stable isotope values of *S. luridus* and *S. rivulatus* varied considerably among Locations in the Cyrenaica coast, when compared to the locations in the Red sea. The two species seem to occupy a border "space" in the new habitats (Cyrenaica coast), both species increases the trophic level when moved into the Cyrenaica coast, while in the Red Sea they appear more tightly clustered and well-segregated. Despite the large variation in *Siganus* species for δ 13C and δ 15N values between *S. luridus* and *S. rivulatus*, but show no relationship with either total length.

Information about trophic and feeding habits of *S. luridus* and *S. rivulatus* in the Cyrenaica coast can allow comparisons between trophic patterns between the Mediterranean and the Red Seas, and to speculate about the fate of these species in their new environments. It can be hypothesized that both species have changed their diet in the Cyrenaica coast compared to the populations in the original habitats. Even though the data available for the Cyrenaica coast is not extensive and lacks information on seasonality, the current findings provide a first insight into the invasive species in Cyrenaica coast and food web, following the invasion by use the stable isotope analysis.

3.1. Introduction

The deliberate or inadvertent introduction of invasive species represents an essential stressor for ecosystems marine biological communities (Grosholz, 2002; Bax *et al.,* 2003). Invasive species can have significant natural effects, such as the escalation of changes in patterns of distribution, abundance and diversity of local species (Claudet and Fraschetti, 2001). The understanding of the ecosystems marine effects of invasive species requires detailed data about trophic ecology.. Assessing the connection between species invasion and food availability add to seeing how and why colonization by invasive species succeeds. Invasive species are one of the main stressors of marine biological communities. Several studies document food web effects of invasive fish, which are likely to result in changes in trophic structure (D'Antonio and Hobbie, 2005; Carvalheiro *et al.,* 2010). A few invasive species may be able to change the trophic web by being highly dominant or competing with native species.

Platt and Denman (1978) argue that 'the structure of community in marine ecosystems varieties came about because of trophic interactions'. Learning the trophic interactions of fish is critical for a comprehension of the species interrelationships occurring in a marine biological system (Odum, 1953). Invasive species can modify trophic ecology structure and change ecosystem-level functioning, but it is often unclear how these invasive species may affect the life history of local species, as invasions create new links altering interspecific relationships at the different trophic levels in the new habitats.

Despite the fact that the biological impacts of trophic disruption can be detected shortly after the invading species has settled, more progressive impacts may take a very long time to show and would thus require long haul information for evaluation for impact of invasive species in the environment. There is a pressing requirement for long term studies that explore natural changes in the trophic structure of a habitat affected by invasions. One underappreciated source of variation regarding invasive species concerns dietary flexibility and shifts in trophic position between native and invasive populations and this form of ecological plasticity may enhance invasion success in a number of ways. Incomplete dietary information greatly hinders an understanding of the community-wide effects of invasions.

The Mediterranean Sea is the most invaded marine region of the world, providing us with a unique unplanned experiment to understand how biological invasions are affecting food web properties in the Mediterranean ecosystems. The opening of the Suez Canal in 1869 has enabled the passage of hundreds of taxa from the Red Sea (Galil 2009 and Zenetos *et al.,* 2012). These organisms, known as Lessepsian migrants (Por, 1978), comprise 90 fish species recorded so far in

the Mediterranean Sea (Golani *et al.*, 2010). The Lessepsian migration has visibly accelerated in recent decades (Ben Rais Lasram and Mouillot 2009; Golani 2010). It is very likely that this large scale invasion, has been reshuffling the overall Mediterranean food web. As invasive species create new links altering interspecific relationships at the different trophic levels. As an example, the native herbivorous fish *Sarpa salpa* is considered to be outcompeted by the rabbitfish *Siganus rivulatus* along the easternmost sectors of the Mediterranean Sea (Bariche *et al.*, 2009).

Siganidae constitute a family of herbivorous fish throughout the Indo-West and Pacific Oceans (Woodland, 1983), feeding on seagrass and the majority of available algae. The *Siganus* species need a big quantity of algal food to assure their biological activities. In addition to algal food, they can feed accidentally on some non-digestible substances such as mollusc shells and other invertebrates attached to algae. The diet and nature of food habits of Siganids have been the subject of many investigations in several studies and feeding geographical areas such as the studies on the diet of *S. rivulatus* and *S. luridusin* the Red Sea (their original habitat; Lundberg and Lipkin, 1979; Lundberg, 1981) and in the eastern Mediterranean (Lundberg, 1980; Stergiou, 1988; Lundberg and Golani,1995; Lundberg *et al.*, 1999).

3.2. Stable Isotope Analysis (SIA)

Stable isotope Analysis (SIA) represent a relatively novel tool for the investigation of trophic connections in marine biological communities (Pinnegar and Polunin, 2000), and an intense approach to delineate the trophic ecology of an organism, upon which ecological niche research relies (Newsome *et. al.* 2007). This procedure offers long haul evaluations of the type of prey that have been incorporated into predator tissue. It has been utilized to reconstruct animal diets (Hobson *et al.*, 1994), to determine patterns of resource allocation to reproduction; to track animal migration and to assess the flux of materials from the sea into terrestrial food webs also to assign trophic levels and to determine the structure of food webs (O'Brien *te al.*, 2002 ; Ben-David *et al.*, 1998 ; Hobson, 1999 ; Post, 2002; France, 1995). Further, increased interest in the utilisation of stable isotopes has been used to research the architecture of the marine food webs (Deegan and Garritt, 1997).

The stable isotope technique depends on the fact that the atoms that make up living beings are derived from the atoms of their food (Hessler *et al.*, 1993). Stable isotopes, in this way, reflect an integrative record of the nourishment that has truly been absorbed by the organism over an extended period prior to sampling. Numerous substance components can have more than one isotopic type of contrasting atomic mass. Examination of the stable isotopic proportions of the different types of elements, such as oxygen, hydrogen, sulfur, etc. can provide insights into the ecology of the

individual/species (Crawford *et al.*, 2008), however the two most ordinarily utilized are the isotopes of carbon (C) and nitrogen (N). The ratio between 13C to 12C (annotated as δ13C) informs on the sources of food types (Wada, 2009); while the nitrogen ratio (δ15N) can be utilized to gauge trophic level, in light of the fact that the d15N of a consumers is commonly enhanced by 3– 4‰ in respect to its eating regimen (DeNiro and Epstein, 1981; Minagawa and Wada 1984; Peterson and Fry, 1987). Thus 15N has been utilized as a part of many examinations as a marker of the trophic level of a creature through its life cycle (Beaudoin *et al.*, 1999; Vander Zanden and Rassmussen, 1999), while 13C is considered to give data on the materials at the base of the nourishment web (Harrigan *et al.*, 1989). Isotopic proportions of carbon and nitrogen have been successfully utilized to depict trophic connections in Mediterranean marine biological communities (Deudero *et al.*, 2004; Pinnegar and Polunin, 2000).

This technique depends on the preface that carbon and nitrogen isotope proportions of a living being mirror the isotopic estimations of the food consumed, hence allowing definition of trophic positions and flow in natural communities (Post *et al.*, 2000). In any case, the isotopic mark of a consumer alone isn't for the most part adequate to deduce trophic position or carbon source without a suitable isotopic benchmark. Only by signature of a consumer alone is not generally sufficient to infer trophic position or carbon source without an appropriate isotopic baseline it is possible to illuminate trophic connections that would otherwise be hard to measure.

3.2.1. Isotopic Dietary Models

Carbon and nitrogen exist in nature in two stable forms. The lighter forms, 12C and 14N, are more common than the heavier isotopes 13C and 15N and it is convenient to refer to the concentrations of the heavier isotopes as a ratio in $^{\delta}$ notation as parts per thousand (‰) as follows:

$$^{\delta}X = [(Rsample / Rstandard) - 1] \times 1,000$$

where (X) is 13C or 15N and R is the corresponding ratio 13C/12C or 15N/14N. These values are measured using a mass spectrometer to precisions typically of the order of 0.1 to 0.3‰.

3.2.2. Carbon

The relative abundance of the steady isotopes of carbon, 13C and 12C, fluctuates typically between various biomes and biological communities e.g. amongst plants and distinctive photosynthetic pathways (DeNiro and Epstein, 1978; Smith and Epstein, 1971) or between terrestrial and marine situations.

The carbon isotope piece of both bioapatites (the mineral part of skeletal tissues) and collagen (the bone protein) are straightforwardly identified with the dietary isotopic sources of info. Accordingly, the examination of these tissues can give data around a creature's eating routine and along these lines about the bolstering nature of animal types, locally accessible plant species and the nearby condition (Balasse and Ambrose 2005; Balasse *et al.*, 2005; Hedges *et al.*, 2004; Hoppe *et al.*, 2006; Richards and Hedges, 2003). Dietary carbon isotope contributions from all food sources are recorded in the body tissues of feeders, with the normal carbon isotopic organization of a creature's tissues mirroring the normal carbon isotopic piece of its diet.

3.2.3. Nitrogen

The study of two of the naturally occurring isotopes of nitrogen (15N and 14N) is also a useful tool in physiological and biochemical investigations. Stable nitrogen isotope ratios, (15N to 14N, expressed as δ15N) can also vary spatially, but are much more useful as a means for determining the trophic level at which an animal is feeding.

3.2.4. Stable isotope analysis for invasive species

Stable isotopes analysis (SIA) represents to a normally utilized device for the investigation of trophic connections in marine ecosystems (Pinnegar *et al.*, 2003), and a solid approach to delineate the trophic properties of organisms (Newsome *et al.*, 2007). Stable isotopes have been utilized as an effective complement/replacement for gut-content analysis (Pinnegar and Polunin, 2000). This technique can help clarifying the trophic role of an invasive species, and its likely impact on the trophic web of the newly-invaded habitat.

Stable isotope analysis (SIA) can significantly reduce field work effort, as it is able, after a solitary catch occasion, to provide data on trophic ecoloyg that span a wide time interval. Layman *et al.*, (2005) mention that the stable isotope ratios are most informative when used in conjunction with stomach content analyses. The results of stable isotope analysis may provide minimal insight into food web structure and the functional role of invasive species fish in the new ecosystems. Stable isotope analysis is increasingly being used to improve interpretation of feeding studies and marine food webs, particularly for invasive species.

A major advantage of stable isotope analysis approaches is that they provide temporally integrated information may indicate 6 months on dietary habits for the invasive species, reflecting foods that are actually assimilated by the consumer. Often it is difficult to identify all of the items within stomach contents, and there are associated uncertainties regarding to their classification due

to the variable feeding habits of invasive species in new ecosystem where basal food sources may change markedly (Rudnick and Resh,2005). Many gut content analysis studies sampled line-caught fish, which are likely to leave the gut empty. Prey may also be digested quickly and thus be under-representation in gut content analyses, while stable isotope analysis likely results in a more comprehensive estimate of predator diets than gut content analysis (Madigan *et al.*, 2012). Since various tissue sorts are supplanted at various rates, the proteins inside them will be integrated at various rates. For instance, stable isotope marks from liver cells mirror the creature's eating routine over earlier days, those of muscle mirror the eating regimen over going before weeks to months, and those of hard parts for even longer (Kurle, 2009).

Invasive species regularly demonstrate high versatility of trophic width (Hayes and Barry 2007). SIA can likewise help with detailing a reaction to intrusions. By understanding the nourishment utilized and areas from which it has been acquired. The immigration of Red Sea organisms through the Suez Canal has caused dramatic alteration of Mediterranean Sea marine communities; however changes at the level of food and feeding habits interactions remain poorly understood (Fanelli *et al.*, 2010). Currently, invasive species provide 'natural' experiments for understanding food web interactions and functional responses in biological communities (Turner *et al.*, 2010). Lesssepsian invasion are paramount to changes to biodiversity in Mediterranean Sea, as reflecting a shifts in diet. Although nutrition studies for lessepsain migration are limited by poor empirical descriptions and depends on traditional methods.

Information about trophic and feeding habits of *S. luridus* and *S. rivulatus* in the Cyrenaica coast can allow comparisons between trophic patterns between the Mediterranean and the Red Seas, and to speculate about the fate of these species in their new environments. It can be hypothesized that both species have changed their diet in the Cyrenaica coast compared to the populations in the original habitats. Even though the data available for the Cyrenaica coast is not extensive and lacks information on seasonality, the current findings provide a first insight into the invasive species in Cyrenaica coast and food web, following the invasion by use the stable isotope analysis. In this study, stable isotope ratios $\delta^{15}N$ and $\delta^{13}C$ were used to quantify trophic relationships for two lessepsian species in Cyrenaica coast, as well as comparing to its native habitats in Red sea. A total of 394 specimens belonging to two *Siganus* species and 9 specimens for algae and sea grasses were processed and send to University of New Brunswick, Canada for analysis.

Table 3. Most reported macrophytes in the stomach content of *Siganus luridus* and *rivulatus* in the Mediterranean Sea (√: occurrence).

Macrophytes	***Species***	
	S. luridus	*S. rivulatus*
Chlorophyta		
Anadyomene spp	√ [1]	*X*
Bryopsis spp	*X*	√ [2,3,4]
Caulerpa racemosa	√ [1]	*X*
Chaetomorpha spp	√ [5]	*X*
Cladophora spp	√ [1,5]	√ [2,3,4]
Codium spp	*X*	√ [1,4]
Enteromorpha spp	*X*	√ [2,3]
Ulva spp	√ [3,4,7]	√ [1,2,3,4,6,7]
Rhodophyta		
Corallina spp	*X*	√ [6]
Gelidiella spp	√ [3,4]	√ [3,4]
Gelidium spp	√ [5]	√ [1,4]
Hypnea spp	*X*	√ [4,7]
Jania spp	*X*	√ [1,2,3,4]
Kallymenia spp	√ [5]	*X*
Laurencia spp	√ [4]	√ [4]
Peyssonnelia spp	√ [3,4]	√ [7]
Polysiphonia spp	√ [1,3,4,5]	√ [1,2,3,4]
Rytiphlaea spp	√ [5]	√ [1]
Sphaerococcus spp	√ [1]	*X*
"Filamentous red algae"	√ [1]	√ [1]
Phaeophyta		
Colpomenia spp	√ [4]	*X*
Cystoseira spp	√ [4,5]	√ [1,6,8]
Dictyopteris polypodioides	√ [4,5]	*X*
Dictyota spp	√ [5]	√ [8]
Dilophus spp	√ [4,5]	√ [1]
Ectocarpus spp	√ [1]	√ [2,3,4]
Halopteris spp	√ [1,3,4,5]	√ [1,2,3,4]
Padina spp	√ [1,3,4,5]	√ [1,4]
Sargassum spp	√ [1,3,4]	√ [1,4,6]
Spatoglossum asperum	√ [4]	√ [2,3,4]
Sphacelaria spp	√ [1,3,4,5]	√ [2,3,4,8]
Taonia atomaria	√ [4]	√ [2,3,4]
Magnioliophyta		
Posidonia oceanica	√[6]	√[6]

[1]Lundberg *et al.*, 1999; [2]Lundberg, 1981; [3]Lundberg and Lipkin, 1993; [4]Lundberg and Golani, 1995; [5]Stergiou, 1988; [6]Dowidar et al, 1992; [7]Lundberg and Golani, 1993; [8]Karagitson *et al.*, 1986

Information about feeding habits of *S. luridus* and *S. rivulatus* in the Mediterranean allows to investigate changes in the diet compared to the original habitats and to speculate about the fate of these species in their new environments (Table 3, fig.38). In the Red Sea *S. luridus* consumes mainly large tough brown algae (Lundberg and Golani, 1995), such as *Lobophora variegata* (Dictyotales), *Cystoseira myrica* and *Sargassum spp* (Lundberg and Lipkin, 1979). The red algae contribute more than half to the diet of *S. rivulatus* (Lundberg and Lipkin 1979, Lundberg and Golani 1995), with fleshy and soft taxa such as *Laurencia spp, Hypnea spp, Champia irregularis* and *Digeneasimplex* are selected most frequently. *Phaeophyceae* and *Chlorophyta* are also an important part of the diet (Lundberg and Golani 1995). El-Mor *et al.*, (2002) observed that *Siganus* species feed mainly on species of green algae increased 50.2%, then the diatoms by 7.7% and then to sediment. The severity of trophism the *Siganus* species up to the highest rates of 100% the winter and spring, where the availability of food.

The diet of *S. rivulatus* was found to be more diverse compared to *S. luridus*, 39 algal and seagrass taxa were found in stomach contents from the gulf of Elat (Lundberg and Golani 1995). *Siganus luridus* shows a stronger preference for brown algae while *S. rivulatus* feeds on a broader range of species and green and red algae play a more pronounced role in the diet. This might be due to the specialised morphology of the alimentary tract which may provide *S. luridus* with a greater ability to utilize coarse brown algae (Lundberg and Golani, 1995).

Most studies show that even though certain food sources are clearly prioritized, members of all three major macroalgae groups are consumed at different times. Both species are able to utilise a broad range of food sources and can switch between preferred groups according to availability, e.g. between seasons (Table 4). Most genera of macroalgae that *Siganus* species feed on occur in both the Red Sea and the Mediterranean, so the main difference they face in their new environments with regards to their diet is different algal community structures and abundances, as well as different seasonality.

The differences in food preferences between the two species has also not changed considerably in the new environment: *S. luridus* shows a stronger preference for brown algae while *S. rivulatus* displays a broader range of targeted food items and red and green algae play a more pronounced role in the diet (Shakman *et al.*, 2007).

Shakman *et al.* (2007) reported that macroalgae and *Posidonia oceanica* are the dominant constituents of the diet of both species, where they explained that, the differences in Gastro-Somatic Index (Gastro Somatic Index is a useful mothed and an efficient way for comparing food consumption during period and for determining the environmental factors and physiological effects on trophic ecology for organisms) values between the two species were

significant in the Libyan coast, where were highest values in the summer and autumn, the ammplitude was increased for *S. rivulatus* than *S. luridus*. The mean Gastro-Somatic Index values ranged between 6.1% and 14.5% for *S. luridus*, while, ranged between from 9.1% to 21.3% for *S. rivulatus*. The Gastro-Somatic Index started to increase in early spring with highest values in summer and autumn before dropping again in winter. The amplitude was lower for *S. luridus* than *S. rivulatus*. A small drop was observed in July for *S. rivulatus*, and in September for *S. luridus.* According to the same study, as preferred food categories of *S. luridus* are *Phaeophyceae* and *Chlorophyta* 60.1% and 55.3%, respectively, followed by *Posidonia oceanica* 43.7% and *Rhodophyt*a 39.9%. While it were, the food preferences of *S. rivulatus* were quite similar, with *Chlorophyta* being the preferred food category 50.6%, followed by *Phaeophyceae* 38.6%, *Rhodophyta* 36.7% and *Posidonia oceanica* 33.5%. Overall, both species show very similar preferences, with the only differences that *S. rivulatus* feeds less on *Phaeophyceae* and *Posidonia oceanica* than *S. luridus.*

More detailed of this previous study, the Occurrence Frequency illustrates that *S. luridus* feeds mainly on *Phaeophyceae* in spring and summer 85.1% and 63.5%, respectively, on *Chlorophyta* in autumn 84.7%, and mainly on *Rhodophyta* 97.0% and *Phaeophyceae* 68.3% in winter. *Siganus rivulatus* feeds mostly on *Chlorophyta* in spring and summer 58.3% and 58.3%, respectively and on *Phaeophyceae* in autumn and spring 55.6% and 50.0%, respectively, and on *Rhodophyta* 65.3% in winter. In general that, the *Rhodophyta* is only an important part of the diet in winter, while seagrass consumption is important in all seasons and does not change much over the year for both *Siganus* species (Table 5).

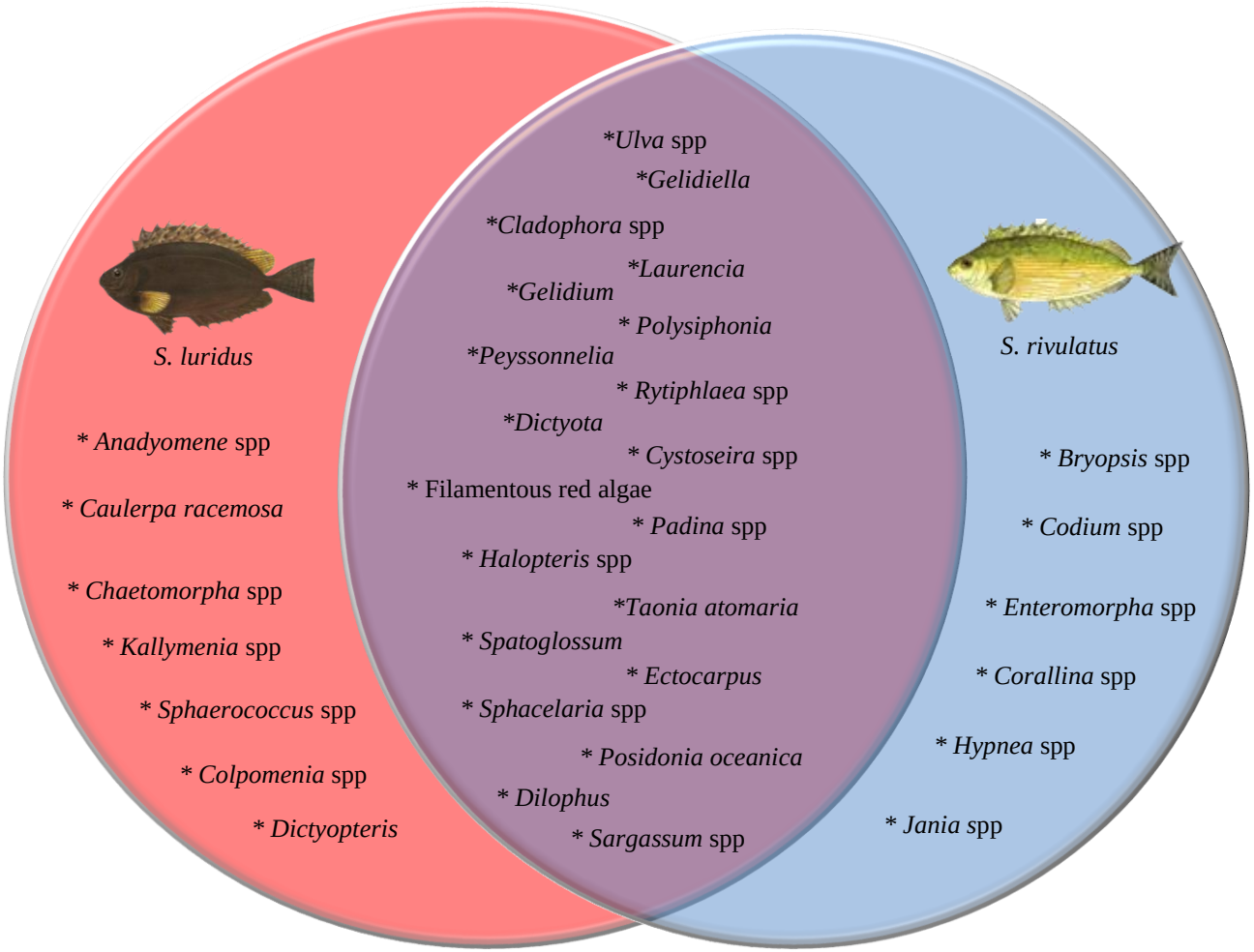


Figure 40. Trophic ecology (algae-seagrass) for *S. luridus* and *S. rivulatus* in the Mediterranean Sea.

Table 4. Seasotnal variation in the most common macrophytes grazed by *Siganus luridus* and *S. rivulatus* in the Mediterranean Sea.

	Season	Macrophytes		
			Stergiou, 1988	Lundberg *et al.*, 1999
Siganus luridus	Winter	Chlorophyta Rhodophyta Phaeophyceae	*Dictyota spp* *Cystoseira spp* *Dictyopteris polypodioides*	
	Spring	Chlorophyta Rhodophyta Phaeophyceae	*Dictyota spp* *Sphacelaria spp*	*Ulva spp* *Sargassum spp* *Padina spp*
	Summer	Chlorophyta Rhodophyta Phaeophyceae	*Dictyota spp* *Sphacelaria spp* *Cystoseira spp*	
	Autumn	Chlorophyta Rhodophyta Phaeophyceae	*Gelidium spp* *Cystoseira spp* *Padina spp* *Dictyota spp*	*Sargassum spp* *Padina spp* *Sphacelaria spp*
Siganus rivulatus			Lundberg and Lipkin, 1993	Lundberg *et al.*, 1999
	Winter	Chlorophyta Rhodophyta Phaeophyceae	*Jania spp* *Polysiphonia spp* *Sphacelaria spp*	
	Spring	Chlorophyta Rhodophyta Phaeophyceae	*Ulva spp*	*Ulva spp* *Enteromorpha spp.* *Cladophora spp.* *Cladophoropsis membranacea*
	Summer	Chlorophyta Rhodophyta Phaeophyceae	*Jania spp*	
	Autumn	Chlorophyta Rhodophyta Phaeophyceae	*Polysiphonia spp* *Sphacelaria spp*	*Sphacelaria spp* *Sargassum spp* *Padina spp*

Table 5. Algal trophic items for *S. luridus* and *S. rivulatus* in the Libyan coast (from Shakman *et al*, 2007). *Posidonia oceanica* was found in both species

***Siganus* species**	**Chlorophyta**	**Rhodophyta**	**Phaeophyceae**
Siganus luridus	*Caulerpa racemosa* *Codium* spp. *Cladophora* spec. *Dasycladus vermicularis* *Ulva spp.*	*Asparagopsis armata* *Hypoglossum hypoglossoides* *Polysiphonia* spp. *Corallina officinalis* *Contarinia squamariae*	*Sauvageaugloia griffithsiana* *Dictyota* spp. *Padina* spp. *Taoina/Spatoglossum* *Cystoseira* spp. *Sargassum* spp. *Halopteris filicina.* *Sphacelaria* spp. *Stypocaulon scoparium*
Siganus rivulatus	*Codium* spp. *Cladophora* spp. *Dasycladus vermicularis* *Ulva spp.*	*Asparagopsis asparagoides* *Antithamnion* spp. *Griffithsia cf. opuntioides* *Heterosiphonia cf. crispella* *Polysiphonia* spp. *Corallina officinalis* *Jania rubens* *Contarinia squamariae* *Rhodophyllis* spp. *Botryocladia* spp.	*Dictyota* spp. *Taoina/Spatoglossum* *Giffordia* spp. *Sauvageaugloia griffithsiana* *Sargassum* spp. *Halopteris filicina* *Sphacelaria* spp. *Stypocaulon scoparium*

Over the most recent two decades, the trophic ecology habits of the *Siganus* species have been widely studied in the eastern Mediterranean Sea (Golani, 1998;Azzurro and Andaloro, 2004;Azzurro *et al.*, 2007 and Bariche, 2006) but no information is available from the Libyan coas except (Shakman *et al.*,2007), so that specific studies are highly required. In reality, one of the real constraints in addressing ecological questions is the absence of data.

Several of invasive species can fundamentally change food web structure in nutrient dynamics. (Wilcove *et al.*, 1998; Wetzel, 2001). In marine environments in the Mediterranean Sea, much attention has been dedicated to spread and distribution of invasive species , but considerably less is known about the competition of invasive with native species and effects of invasive fish for food web. The impacts of invasive species can extend to affect change food web structure of ecosystems, also likely to result in changes in trophic structure in ecosystems. The response of *Siganus* species to adapt in Mediterranean ecosystems may be its ability to exploiting various habitats. Thus, Iwould expect those two *Siganus* species to provide different levels, including invasive plants.

3.3. Aims

A number of studies have been conducted in Libyan waters on trophic ecology patterns of Libyan fish, concentrated on stomach content analysis only (Shakman *et al.*, 2007, Ben Abdallah *et al.*, 2005). In this study stable isotope analysis was used to compare the trophic ecology of the two *Siganus* species under investigation in different locations in the Cyrenaica coast and the Red sea. I was interested in understanding whether invasive species changes their feeding habits in the invaded Cyrenaica coast. I chose stable isotope analysis because it provides a rapid and relatively cost-effective assessment of trophic structure (Fry, 1991) and an integrated measure of changes in lower trophic levels (Layman *et al.*, 2007). Due to isotope fractionation, 15N accumulates as trophic position increases, whereas carbon undergoes minimal fractionation and can be used to track basal resources (Fry, 1991).

The aim study is to evaluate to what extent the stable isotope analytical methods can provide us with knowledge of the structure and functioning of invasive species in Cyrenaica coast and understanding the feeding of invasive species.

3.4. Material and methods

3.4.1. Samples collections

The samples were collected 2014 and 2015 by Fishermen from the Libya coast (Cyrenaica) and the Egypt coast (Red sea). *Siganus rivulatus* and *S. luridus* were collected from the Cyrenaica coast Six regions (Al-haniya, Susa, Ain Al-Gazala and Tubruq) and Egypt coast two regions (Hurgadae and Al-Ture). The tissues were taken from fish samples had been dried at a temperature of 60° C for 24 hours subsequently they had been crushed and placed in plastic bags for the purpose of sending it to Canada for the required analysis (Fig. 41). It is worth mentioning that samples of existing seaweed and algae have been gathered in the location of gathering the samples whether on the coast of Cyrenaica, and the coast of the Red Sea. They were treated by draining them on temperature 60 ° C for 24 hours and then they were crushed and stored in plastic bags. Samples were analysed at the centre of Canadian Rivers Institute Department of Biology University of New Brunswick.

Figure 41. Plant and algae samples and fish muscles were dried at 60 C for 24 hour for the stable isotope analysis

3.4.2. Carbon and Nitrogen Methodology

Samples are weighed into tin capsules and loaded into either a PN150 or Costech Zeroblank autosampler. Samples are converted to gases by combustion in either a Carlo Erba NC2500 or Costech 4010 Elemental Analyzer (EA).

For measurements of $\delta^{13}C$ and $\delta^{15}N$, samples are analyzed using either a Delta Plus or a Delta XP continuous flow isotope-ratio mass spectrometer (CF-IRMS) (Thermo- Finnigan; Bremen, Germany), interfaced to the EA via the Conflo II or Conflo III, respectively. Combustion occurs in a quartz tube filled with chromium oxide and silvered cobaltous oxide at a temperature of 1050ºC (NC2500) or 1000ºC (Costech 4010).

A second quartz tube set at 650ºC is filled with fine copper wire and used for the reduction of nitrogen oxides (NxOx) to N2. CO2 and N2 peaks are separated while passing through a 4m GC column (NC2500) held at 50ºC or a 3m GC column (Costech 4010) held at 40ºC. A water trap of magnesium perchlorate & silica chips is located prior to the GC column to remove water. Stable isotope measurements are reported as isotope delta δ in parts per thousand (‰) relative to the international standard: Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric air (AIR) for nitrogen. Isotope values are normalized using secondary standards: NICOTINAMIDE, BLS, and SMBM for animal tissues; and CMS, AQM, SPL and EPS for sediments and plant material. All of these standards were calibrated against IAEA standards. See below for standard descriptions.

3.4.3. Isotope analyses

Isotopic analyses were performed on muscle tissue samples of *Siganus luridus* and *S. rivulatus* from six site and macrophytae (chosen on from the Cyrenaica coast and the Red sea). According to each fish species, I analysed six individuals of the same size (ANOVA $P>0.05$) Analysis of Variance (ANOVA) is a statistical method used to test differences between two or more means. For each species of macrophytae, I analysed two samples taken from a composite sample of specimens. Samples of both fish and macrophytae were dried to constant weight at 60°C in a fan-assisted oven before being ground to a fine powder with a mortar and pestle.

The permutational univariate ANOVA was executed based only on stable isotope for the $\delta^{13}C$ values of the same matrix. The bathymetric direction of each group was also explored by regression analysis for $\delta^{13}C$ and $\delta^{15}N$ separately (Anderson et al., 2008). Bathymetric trends of each group were also explored by regression analysis for $\delta^{15}N$ and $\delta^{13}C$ separately. In addition, a permutational multivariate ANOVA (PERMANOVA; Anderson 2008) was

employed on the same matrix to test the significance of differences between the diet of *S. luridus* and *S. rivulatus* belonging to the six sampling locations.Significance was set at p = 0.05 and p-values were obtained using 9999 permutations under unrestricted permutation of the raw data.

3.5. Results

The estimates of stable isotope analysis in *Siganus* species (Table 6), where were the values range of $\delta^{13}C$ were (-16.2 ‰ and -18.4 ‰) for *S. luridus* and *S. rivulatus*, while the values of $\delta^{15}N$ were (5.9 ‰ and 9.7 ‰) for *S. luridus* and *S. rivulatus* respectively in the Cyrenaica coast. On the other hand, the values range of $\delta^{13}C$ were (-13.1 ‰ and -10.1 ‰), while the values of $\delta^{15}N$ were (5 ‰ and 4.1‰) for *S. luridus* and *S. rivulatus* respectively in the Red sea. The highest $\delta^{13}C$ values were found in *S. rivulatus* in Tobruq (-6.7 ‰) and the lowest in Susah *S. luridus* (-21.9 ‰), while the highest $\delta^{15}N$ values were found in *S. rivulatus* in Tobruq (10.8 ‰), and the lowest in the *S. luridus* in Al-Haniya in (2.7 ‰) in the Cyrenaica coast. On another hand, the highest $\delta^{13}C$ values were found in *S. luridus* in Hurghada (-7.2 ‰) and the lowest in Hurghada *S. rivulatus* (-15.9 ‰) and the highest $\delta^{15}N$ values were found in *S. luridus* in AlTor (9.6 ‰) and the lowest in AlTure *S. luridus* (-15.9 ‰).

Table 6. Isotopic composition ($\delta^{15}N$ and $\delta^{13}C$; Minimum, Median and Maximmum) of the two *Siganus* species for the study areas.

Isotope	Min	1st Qu.	Median	Mean	3rd Qu.	Max
$\delta^{13}C$	-21.9	-17.4	-14.4	-14.3	-11.8	-6.6
$\delta^{15}N$	3.65	3.5	5.75	5.56	6.4	8.35

Stable isotope values of $\delta^{13}C$ varied among locations in the Cyrenaica coast and the Red sea (-25.6‰ to -6.5 ‰) while, the values of $\delta^{15}N$ varied between 1.7 ‰ to 8.7 ‰. On another hand, the values for $\delta^{15}N$ in baselines were more extreme (Table 7) than previously reported values, where the values of $\delta^{15}N$ were for baseline were 5.6 ‰, while the values for $\delta^{13}C$ were -13.4 ‰.

Table 7. Isotopic composition ($\delta^{15}N$ and $\delta^{13}C$; Minimum, Median and Maximmum) of baseline (algae and seagrasses) for the study areas.

Isotope	Min	1st Qu.	Median	Mean	3rd Qu.	Max
$\delta^{13}C$	-25.6	-17.03	-11.26	-13.41	-10.38	-6.58
$\delta^{15}N$	2.79	3.19	5.08	5.67	7.22	10.81

The $\delta^{13}C$ values were in *Siganus* species in the Red sea higher than $\delta^{13}C$ values for in Cyrenaica coast, where were range between -7.2 to -15.9, the $\delta^{13}C$ values of *Siganus* species tissue (fig.42).

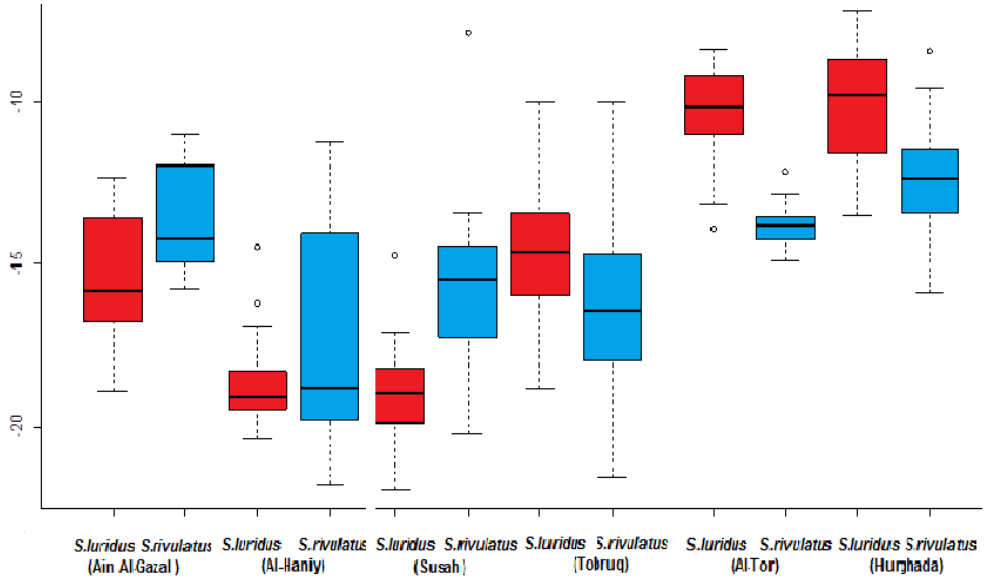


Figure 42. Box – Whisker plots of the fractional trophic level values of Carbon for *Siganus luridus* (Blue) and *S. rivulatus* (Red) by habitat locations. The central box covers 50% of the data, the whiskers extend out to the minimum and maximum values of the data, the vertical line within the box is the median and the black dot is the mean. Numbers in parentheses indicate number of datasets per habitat type.

In the same context, when mentioning the when using length as a covariate to compare differences in weight, it was found that the *Siganus luridus* growth was higher in the Red sea compare to the Mediterranean sea (see Fig.21 in chapter II), as shows there differences are significant between *S. luridus* and *S. rivulatus* and all the locations in both habitats and years. Comparison between nitrogen isotope compositions in scale tissue from *Siganus* species in the Cyrenaica coast and the Red sea, *S. rivulatus* in the Cyrenaica coast clearly fed at higher trophic levels than the *Siganus* species in the Red sea, reflecting changes in trophic level for *S. rivulatus* in the native and novel habitats. On the other hand the *S. luridus* remained at the same trophic

level at all locations except Tobrouq (Fig. 43), on the other hand, the growth of *S. rivulatus* was higher in the Cyrenaica coast compare to the Red sea (see Fig. 22 chapter II).

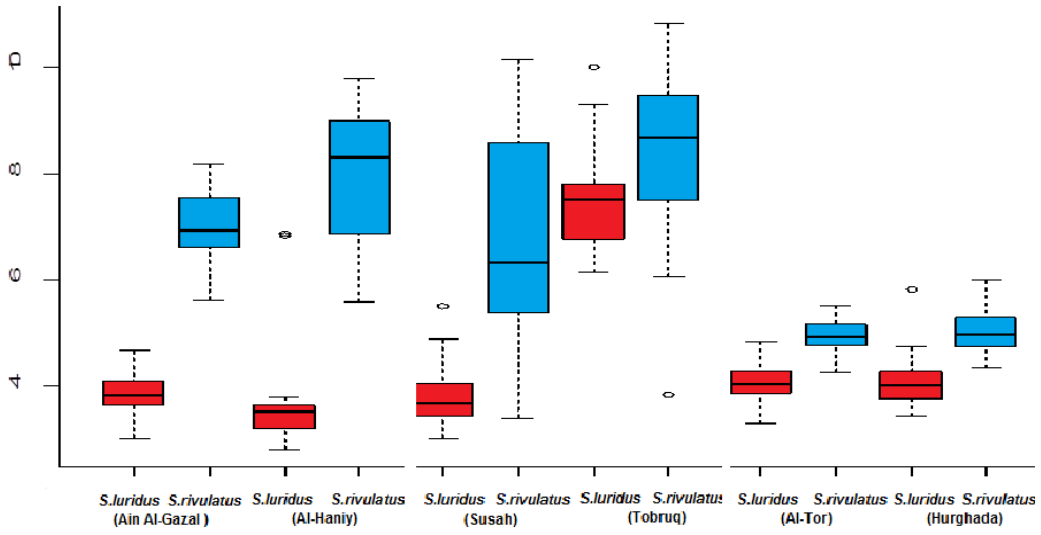


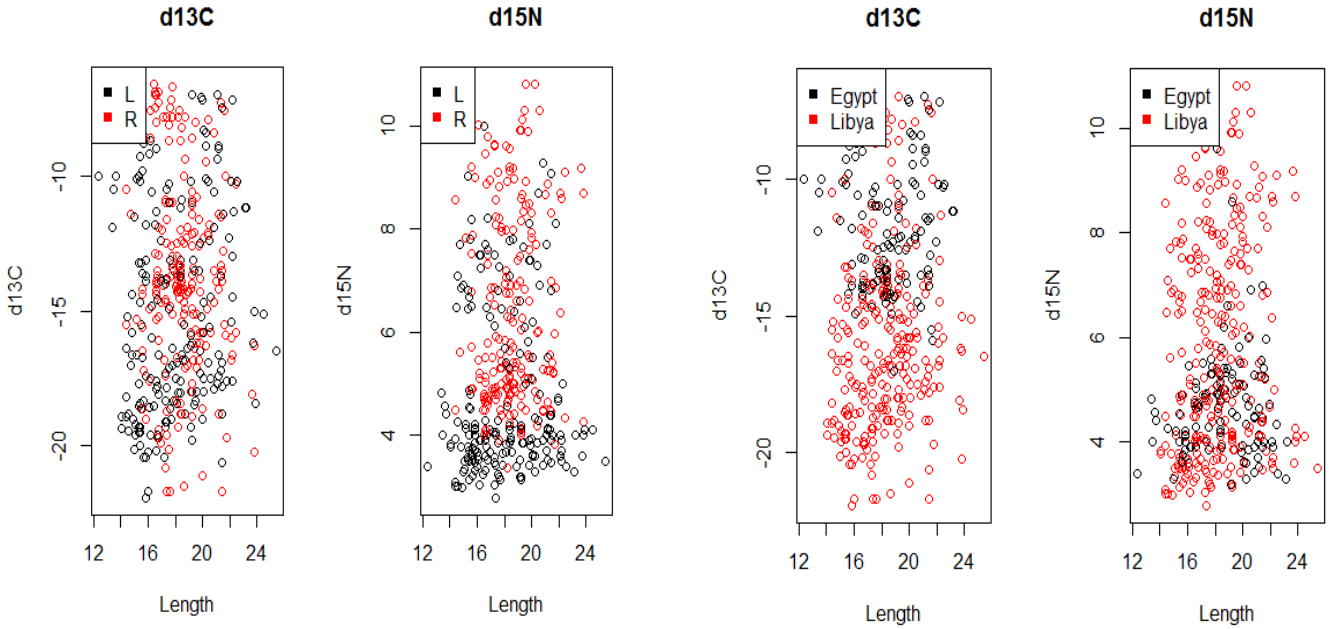
Figure 43. Box – Whisker plots of the fractional trophic level values of Nitrogen *Siganus luridus* (Blue) and *S. rivulatus* (Red) by habitat locations. The central box covers 50% of the data, the whiskers extend out to the minimum and maximum values of the data, the vertical line within the box is the median and the black dot is the mean. Numbers in parentheses indicate number of datasets per habitat type.

Despite the large variation in *Siganus* species for δ 13C and δ 15N values between *S. luridus* and *S. rivulatus*, but show no relationship with either total length (Fig.44), indicate that differences in the total length for *Siganus* species were not always correlated with δ 15N and δ13C. In spite of, there were significant differences between the study sites, also between the years; the differences were less significant between species (Table.8).

Table 8. Summary of ANOVA results.

	Df	Sun Sq	Mean Sq	*F* value	Pr (>*F*)
Locations	5	251.4	50.27	12.871	1.36e-11 ***
Species	1	17.8	17.8	4.55	0.0334 *
Years	1	219.9	219.9	56.3	4.30e-13 ***
Residuals	387	1511.6	3.91		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1



(a) Relationships between δ ^{13}C and δ 15N values and total length for(circle symbols) *Siganus luridus* (**L**) **black** and S. *rivulatus* (**R**) **Red**.

(b) Relationships between δ ^{13}C and δ ^{15}N values and total length for (circle symbols) Egypt, **black** and Libya, **Red.**

Figure 44. Relationships between δ ^{13}C and δ ^{15}N values and total length for *Siganus* species and native and novel habitats.

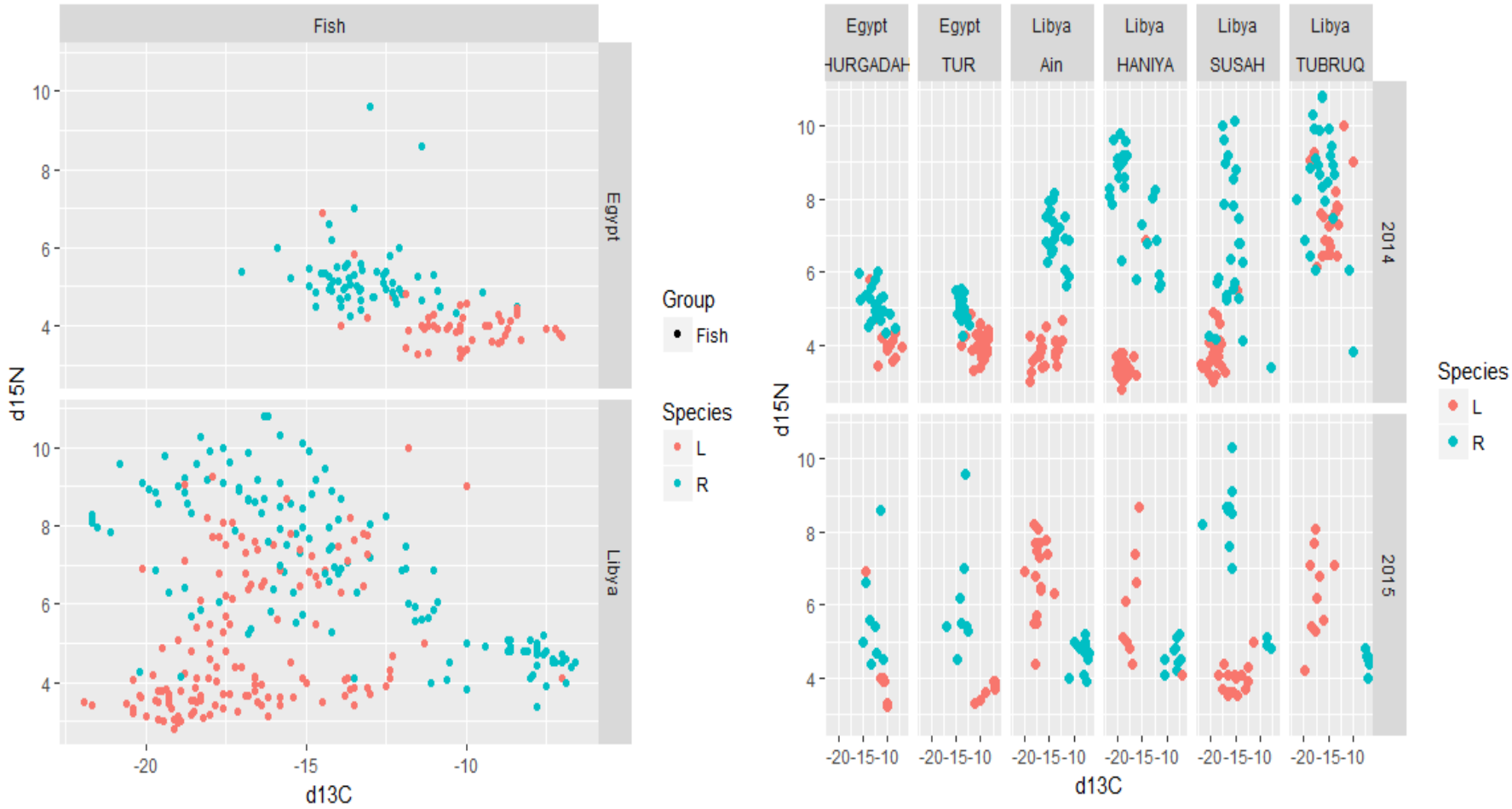


Figure 45. $\delta^{15}N$ versus $\delta^{13}C$ scatter plot of *Siganus* species, collected in the Red Sea and along the Cyrenaica coast illustrating the isotopic signature of *S. luridus* (circle, red) and *S. rivulatus* (triangle, blue) in the study sites

Stable isotope values of *S. luridus* and *S. rivulatus* varied considerably among Locations in the Cyrenaica coast, when compared to the locations in the Red sea (Fig.45). The two species seem to occupy a border "space" in the new habitats (Cyrenaica coast). In the box plots (fig.45) where, both species increases the trophic level when moved into the Cyrenaica coast, while in the Red Sea they appear more tightly clustered and well-segregated. Lack of isotopic niche overlap between *S. luridus* and *S. rivulatus* in the majority of locations in the Cyrenaica coast indicated a predominantly indirect interaction. I suggest that *Siganus* species induced habitat alteration resulting in a reduction in the dietary niche. Therefore, I attribute any changes in the stable isotope values and population metrics of *S. luridus* and *S. rivulatus* to actual diet alteration in the Cyrenaica coast but, based on δ15N values, I conclude that *Siganus* species are feeding at a similar trophic level in the Red sea, this data supports the conclusion that *Siganus* species are generalist feeders. Once *Siganus* species had become established as the dominant fish species in Cerynaica coast, *Siganus* speciess exhibited change reflecting a shift in diet towards higher trophic levels.

Table 9. Summary of PERMANOVA results.

	Df	Sums of Sq	Mean Sq	*F* Model	R2	Pr (>*F*)
Years	1	344.1	344.1	62.91	0.049	0.001***
Sea	1	1253.3	1225.3	229.1	0.18	0.001***
Locations	5	433.9	108.4	19.83	0.062	0.001***
Species	1	433.4	443.4	81.06	0.064	0.001***
Years : Sea	1	144.5	144.5	26.42	0.02	0.001***
Years : Locations	5	205.7	51.42	9.04	0.02	0.001***
Years : Species	1	416.8	416.7	76.2	0.06	0.001***
Sea : Species	1	728.1	728.1	133.12	0.105	0.001***
Locations : Species	5	202.1	50.52	9.23	0.029	0.001***
Years : Sea : Species	1	206.3	206.29	37.71	0.029	0.001***
Years : Locations : Species	5	520.7	130.18	23.8	0.075	0.001***
Residuals	371	2029.1	5.47	0.292		
Total	394	6927.9	1			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

In Ain Ghazala (Cyrenaica coast), there was a difference in δ15N isotope ratio between the two species, in 2014 the trophic level for *Siganus rivulatus* it was higher than trophic level for *Siganus liridus* (Fig.46). While in 2105 the trophic level for *Siganus luridus* it was higher than trophic level for *Siganus rivulatus* (Fig.46). The two species seem trophic level in the Tubruq (Cyrenaica coast) in 2014 (Fig.46), the other hand the *Siganus luridus* in Tubruq higher for trophic level than all locations in Cyrenaica coast. In 2015 the trophic level for *Siganus luridus* in Susah it was low than all locations in Cyrenaica coast (Fig.46). The Trophic level was different in *Siganus rivulatus* in Egyptian locations (Al-Tur and Hurgadah) between 2014 and 2015, where was in 2015 higher from 2014 (Fig. 46).

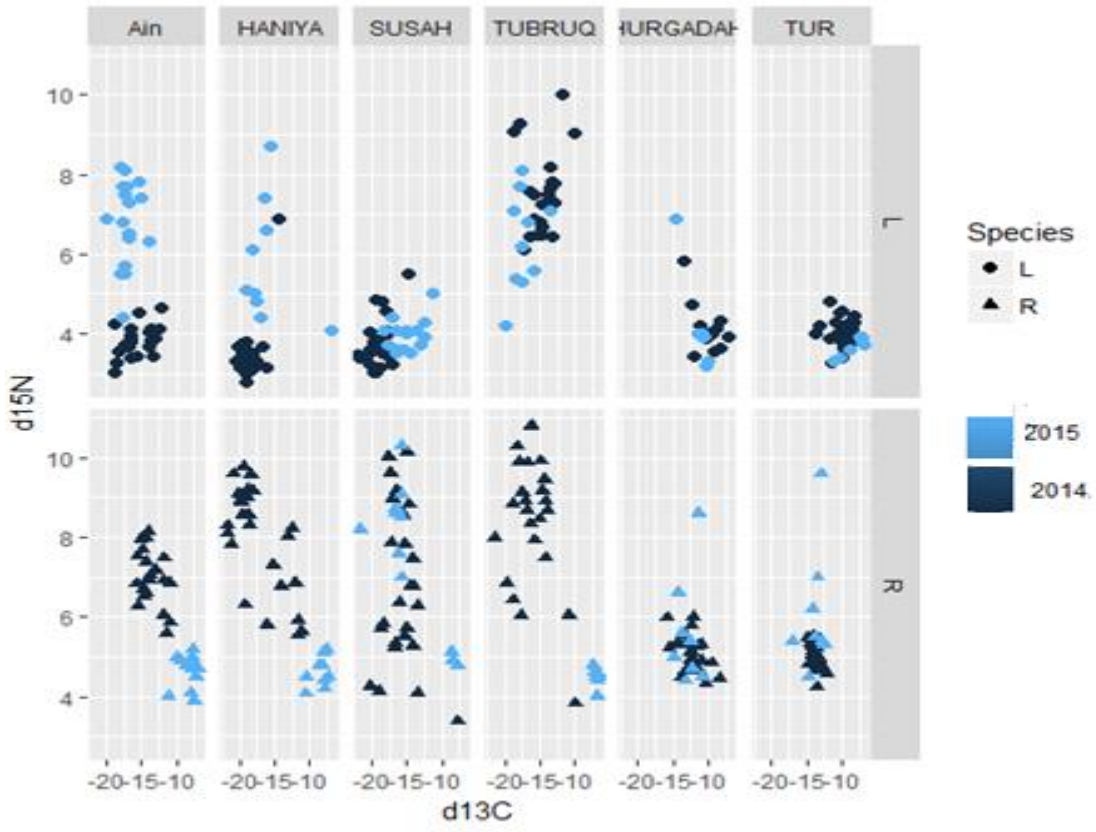


Figure 46. $\delta^{15}N$ versus $\delta^{13}C$ scatter plot of *Siganus* species, collected in the Red Sea and along the Cyrenaica coast illustrating the isotopic signature of *S. luridus* (circle) and *S. rivulatus* (triangle) in the 2014 and 2015.

PERMANOVA pair-wise test showed significant differences in the isotopic ratios of *Siganus luridus* and *S. rivulatus* species, among trophic groups and for the interaction (Table 9). The pair-wise comparisons performed on the interaction ‘‘Species versus Locations versus Years’’ showed significant differences in δ15N– δ13C. There was no overlap between two locations in Cyrenaica coast Ain Al-Gazalh and Al-Haniya, while were clear overlap in two sites Tubruok and Susah. On

the other hand, the Egyptian sites well-segregated a there were no overlap between species at 2014. In contrast, in 2015 there were radical changes of variations in the isotopic composition when comparing the data between locations and years, the trophic level for *S. luridus* was increased compared with 2014 , in contrast the trophic level for the *S. rivulatus* was decreased in the Cyrenaica coast. When compared the isotopic composition of *S. luridus* and *S. rivulatus* in the study areas, both species in the Egyptian sites were very similar for Isotopic composition during 2014 - 2015, while there was fluctuated in the isotope composition between the species in locations in the Cyrenaica coast (Fig. 46)

Table 10. List of species/taxa, their isotopic composition, considered as potential primary food sources for littoral fishes off the coast of Cyrenaica

Sea	**Locations**	**Species**	**δ13C**	**δ15N**	**C/N**	**Group**
Libya	Ain	*Posidonia*	-6.58	3.29	17.91	Algae
Libya	HANIYA	*Cystoseria*	-18.21	-1.09	46.84	Algae
Libya	TUBRUQ	*speciesA*	-7.38	2.81	21.87	Algae
Libya	SUSAH	*SpeciesB*	-25.66	7.09	32.18	Algae
Egypt	HURGADAH	*RedAlgae*	-17.03	1.76	42.17	Algae
Egypt	TUR	*Sargassum*	-10.38	8.98	11.19	Algae
Egypt	HURGADAH	*Sargassum*	-13.03	8.77	9.86	Algae
Egypt	TUR	*SpeciesC*	-11.18	8.45	11.33	Algae
Egypt	HURGADAH	*SpeciesD*	-11.26	8.16	11.06	Algae

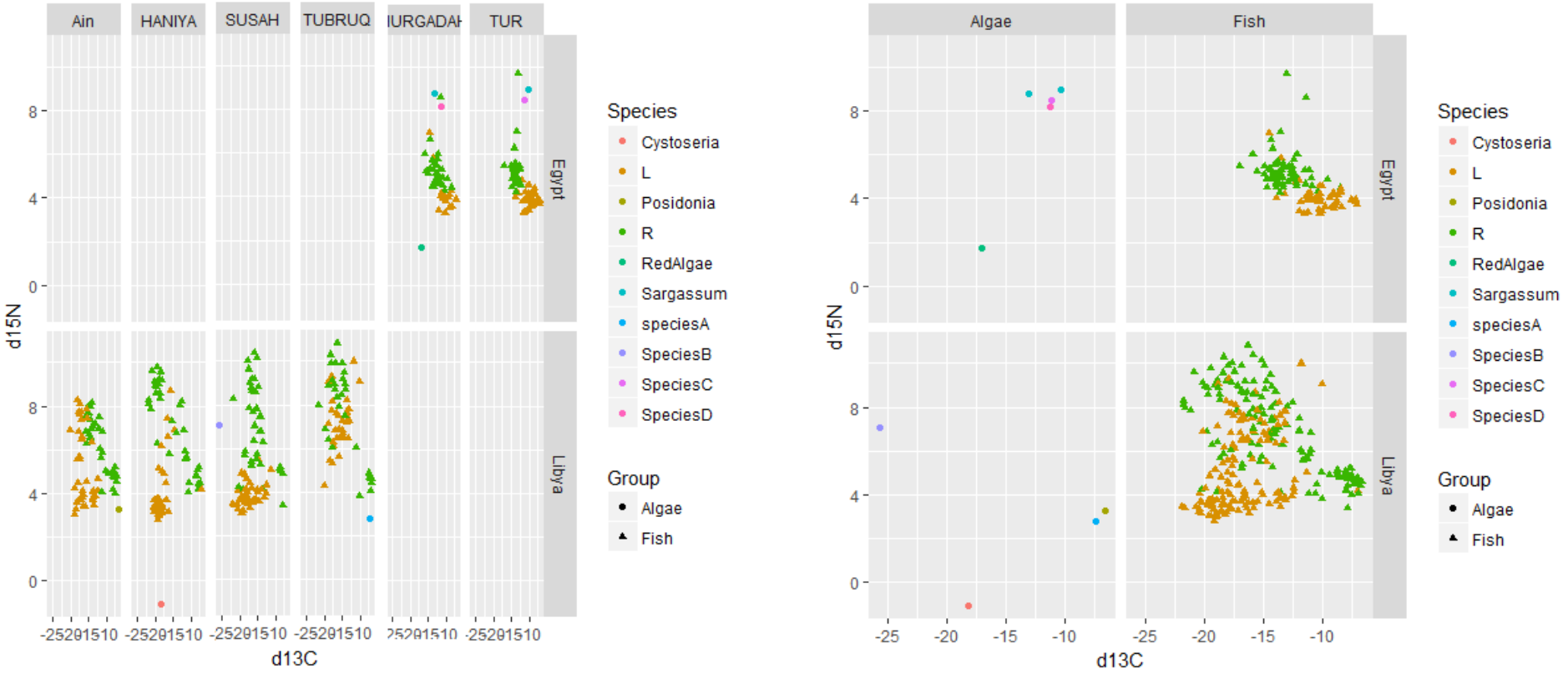


Figure 47. $\delta^{15}N$ versus $\delta^{13}C$ scatter plot of baseline and *Siganus* species, collected of Red Sea and in Cyrenaica coast, illustrating the isotopic signature of fish and algae.

Although data on vegetation from Cyrenaica coast and the Red sea are required in order to assess selectivity In two *Siganus* species, some general conclusions can be drawn based on the previous studies of Shakman *et al.*,(2007) in the Libyan coast and another study at Mediterranean sea (Stergiou,1988; Lundberg *et al.*,1999), also other study by McMahon *et al.*,(2016) in the Red sea. Baselines in the Mediterranean sea and the Red sea are very variable, thus the diet of *Siganus* species are more diverse between different habitats, also both species are feed mainly on algae and seagrass.

In this context, previous data obtained has been used, especially for the baseline in the Red sea and the Cyrenaica coast. The following was observed, the δ15N values of *Siganus luridus* and *S. rivulatus* were higher enriched than expected on the basis of consumed food items in the Red Sea, while the values of $\delta^{15}N$ of the baseline at the Cyrenaica coast at the same level with $\delta^{15}N$ in *S. luridus*.

On the other hand, the values of $\delta^{15}N$ in *S. rivulatus* was higher than baseline. Also, spatial differences were revealed, analysing all the Seas and Species together for both $\delta^{13}C$ and $\delta^{15}N$ values, each Seas can be separated in picture (Fig. 47).

In addition, the lower $\delta^{13}C$ range found in *Siganus* species would suggest a narrower range of food sources in the Red Sea. In contrast, what happened on the coast of Cyrenaica coast was an expansion of the used resources.

Table 11. List of secies/taxa, their isotopic composition, considered as potential primary food sources for littoral fishes off the Red sea by McMahon *et al.* (2016).

Sea	Species	δ13C	δ15N	Groyup
Red Sea	Mangrove	-27.7	1.4	Mangrove
Red Sea	Seagrass	-7.9	-0.3	Seagrass
Red Sea	Seagrass	-5.5	2.4	Seagrass
Red Sea	Seaweed	-14.3	2.8	Seaweed
Red Sea	Seaweed	-7.4	0.4	Seaweed
Red Sea	Seaweed	-10.5	1.7	Seaweed
Red Sea	Macroalgae	-8.2	1.9	Macroalgae

Table 12. List of species/taxa, their isotopic composition, considered as potential primary food sources for littoral fishes off the coast of Cyrenaica by (Fanelli *et al.,* 2015 and Shakman *et al.,* 2007).

Sea	Species	δ13C	δ15N	Group
Mediterranean	*Enteromorpha species*	-17.2	15.9	sea grass
Mediterranean	*Halopila stipulacea*	-18.3	4	sea grass
Mediterranean	*Hypnea species*	-17.8	4.8	red algae
Mediterranean	*Posidonia oceanica*	-13.8	2.4	sea grass
Mediterranean	*Asparagopsis taxiformis*	-31.9	2.5	red algae
Mediterranean	*Caulerpa cylindracea*	-15.6	3.7	green algae
Mediterranean	*Codium bursa*	-10.5	1.3	green algae
Mediterranean	*Ulva sp.*	-20.3	4.1	green algae
Mediterranean	*Colpomenia sinuosa*	-11.7	1.7	brown algae
Mediterranean	*Cystoseira sp.*	-17.5	1.5	brown algae
Mediterranean	*Dictyota sp.*	-14.7	1.5	brown algae
Mediterranean	*Halopteris scoparia*	-22.7	2	brown algae
Mediterranean	*Padina pavonica*	-12.3	2.8	brown algae
Mediterranean	*Amphiroa rigida*	-16.8	3.8	red algae
Mediterranean	*Ceramiales*	-16.4	3.6	red algae

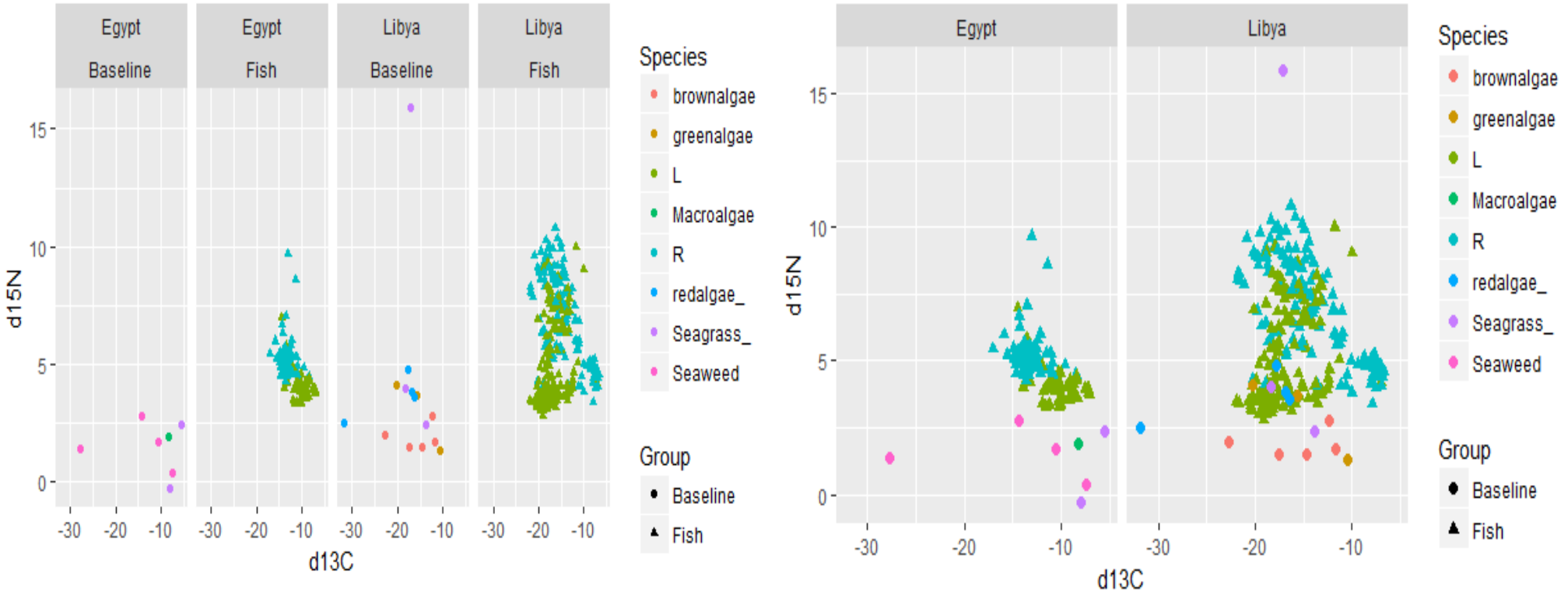


Figure 48. $\delta^{15}N$ versus $\delta^{13}C$ scatter plot of baseline data from previous studies (Fanelli *et al.*, 2015 and Shakman *et al.*, 2007) and isotopic signature of *Siganus* species, collected of Red Sea and in Mediterranean sea.

3.6. Discussion

Our findings showed a very different situation. In facts the higher δ15N values of baseline in the Red sea (see Table.10 and Fig.47). The δ15N of baseline it's higher levels in the food webs, especially two Locations in the Red sea. These points to a displacement *Siganus* species to lower trophic level for baseline in the Reds Sea, also higher δ15N values for *Siganus* species in the Red Sea could indicate a more oriented diet towards organisms of Algae and seagrass sources contains low values of nitrogen. While δ15N values were higher in *Siganus* species than the baseline in Cyrenaica coast that means, the *Siganus* species in the Cyrenaica coast use many baselines than Red sea, hence higher variations between both species in native and novel habitats (fig.47).

The $\delta^{15}N$ values for the baseline at these locations (see Table.10 and Fig.47) may be indicating higher exposure to sewage-derived organic matter. The rise of the $\delta^{15}N$ value in seagrass and algae was consistent with the enrichment of the seagrass and algae with observed the presence of organic resources from human and animals waste (Salvatrice and Mazzola, 2006). Where were collected the samples from sea fishing ports, which there were anthropogenic inputs. Therefore, it appears that unpredictable stable isotope analysis for the baseline, when taken from cited there are anthropogenic inputs

Stable N isotopes provide another means of assessing ontogenetic changes in *Siganus* species diets and the trophic level at which *Siganus* species are feeding. The isotope values of muscle tissue integrate diet over a longer period of time than do stomach contents, and reflect the isotope values of all assimilated prey, rather than the identifiable remains of ingested prey. Stable isotopes provide an integrated signal of diet over space and time, offering an appropriate scale for the study of population niches, but few isotope studies have retained the often insightful information revealed by variability among individuals in isotope values. Our population metrics incorporate such variation, are robust to the vagaries of sample size and are a useful additional tool to reveal subtle dietary interactions among species

In the absence of historical data for *Siganus* species on the native and the Cyrenaica coast for food web, I took into account literature isotopic data of two *Siganus* species in the native and novel habitats. The data from native areas (Red sea) were took from McMahon *et al.* (2016) (Table 11), While the data were taken for the Mediterranean sea (novel habitats),of the study conducted by Fanelli *et al.* (2015) in the Lebanon waters (Table 12). The data from the Mediterranea Sea of the baseline were used the items, its Recorded by (Shakman *et al.*, 2007) in the Libyan coast in order to compare.

The differences between the diets of *S. luridus* and *S. rivulatus* in these and native and novel habitats may be attributed to the differential quantitative composition of the phytobenthic, as well as regard to the distribution and abundance of benthic algae in the waters of the Cyrenaica coast. Seasonal variations in the abundance and availability of the important phytobenthic of *S. luridus* and *S. rivulatus* could be a major factor leading to variations in the diet of this species in the Cyrenaica coast. Where, Shakman *et al.*, (2007) found that in the Libyan coast the *Posidonia oceanica* it's the important part of the diet for the *S. luridus* and *S. rivulatus* in all seasons. The variations changes in the abundance of the phytobenthos agree to a great extent with the seasonal changes observed in the feeding spectrum of *S. luridus* and *S. rivulatus*. The relative abundance of red algae in the area of Cyrenaica Costa increases significantly in the autumn (Diannelidis 1977) and this may explain the increased consumption of red algae by *S. luridus* and *S. rivulatus* in the autumn. also, Shakman *et al.*, (2007) noted that, the *S. rivulatus* targets a broader range of feed items than *S. luridus* in the Libya coast. Barich, (2006) found that the grazing of macrophytes varies in proportion in *S, luridus* and *S. rivulatus*, reflecting the seasonal variations of macrophyte populations. The proportioning also appears to change with fish size, probably in relation to energy needs as well as changing grazing capabilities with relation to age.

While *Siganus luridus* and *S. rivulatus* showed a similar range of trophic level (fig.48), as this overlap seems to be particularly clear for *Siganus* species in the native and novel habitats and strong competitive interactions are expected to occur among them, this is consistent with the previous studies based on stomach contents (Shakman *et al.*, 2007) highlighted a large overlap between the diets of *Siganus* species in the Libyan coast.

As, the d15N of *Siganus* species appeared more enriched in the Cyrenaica coast, as if they were consuming higher trophic level prey compared with the Red Sea, this evidence reinforces the idea of a measurable niche shift of *Siganus* species, Lundberg and Golani,(1995) noted that, the *S, rivulatus* was more diverse than *S. luridus* in Gulf of Aqaba. in addition, Barich, (2006) pointed out to, in the Mediterranean sea, both siganidae have considerably modified their diet to adapt themselves to new algal resources, which differ significantly from those found in the Red sea. Differences in isotopic values resulted for *Siganus* species in native and novel led to change for trophic levels, as Since the process of invasive species by Red Sea Lessepsian species still continues without any sign of decline (Ben-Tuvia, 1985), it seems, as Por (1978) stressed, that the Mediterranean ecosystem allows for additional links in the food web. As mentioned by Stergi, (1988) fishes clearly alter their behavior in the presence of competitors in order to reduce the utilization of similar resource,

According to Pinnegar and Polunin (1999) when the animals consume their food, they become reinforced by heavier isotope ($\delta^{13}C$ or $\delta^{15}N$). As known that the seasons and space its It has a great effect on both diet and feeding behavior of fishes (Deudero *et al.*, 2004). In addition to that, *Siganus* species, showed significant differences also between 2014 and 2015, they as referred to that, the range of variation were clear. Such a discrepancy may suggest that different physiological and ecological factors are involved in fish isotopic composition. The variable temporal contribution of resources may be ascribed to its annual cycle as it reaches the maximum biomass and production in autumn.

The trophic diversity of *Siganus* species in the Cyrenaica coast shows a positive relationship with baseline sources of prey types utilized in the food web in the six locations of Cyrenaica coast. Coupled with the present data, this suggests an increased used of baseline sources by the *Siganus* species, confirming results of previous studies.

I conclude that presence of *Siganus* species was associated with changes in trophic diversity in the Cyrenaica coast but not trophic position, where the trophic position for both *Siganus* species in differents sites in native and novel habitats were similar. Unlike the trophic position, trophic diversity width was significantly different in six locations in the Cyrenaica coast. It is interesting that the expansion of trophic diversity of *Siganus luridus* and *S. rivulatus* in the Cyrenaica coast adds a new potential link to the use of local trophic resources and competitive interactions between this lessepsian migrant and its native ecological analogues. It is possible that *Siganus* species may experience selective pressure for increasing generality to counteract stress of habitat change in the novel habitats in The Cyrenaica coast.

Chapter IV

Comparative phylogeography of the Lessepsian invaders: *S. luridus* and *S. rivulatus*

Abstract

Invasive species are often exposed to new selective pressures, as they try to adapt to a new habitat. The Lessepsian invasion is relatively new and it is was long believed that only a small number of organisms originating from the Red Sea had made their way to settle in the Mediterranean Sea.

Phylogenetic relationships of two rabbitfishes (*S. luridus* and *S. rivulatus*), the Lessepsian species that are important components as primary consumers in Cyrenaica coast, were studied using mitochondrial control region sequence analyses. Samples were collected at four sites in the Cyrenaica coast - Libya and two sites in the Red sea. The samples were gathered in October and November 2014 and August and September 2015. DNA was extracted using a Chelex protocol. The sequences of the control region for *S. luridus* and *S. rivulatus* from the Cyrenaica coast in the Libyan Sea were generated in the lab at Salford University.

Based on the haplotype networks using mtDNA of the *S. luridus* and *S. rivulatus* were well differentiated and did not separate into native population (Red sea) and Mediterranean populations. A genetic differentiation index based on the frequency of haplotypes confirms the absence of genetic differentiation in *S. luridus* and *S. rivulatus*. The Cyrenaica population, Red sea populations, and the Mediterranean population are closely related to each other, suggested by a very low and non-significant FST value. This is likely due to high levels of gene flow between the Red Sea and the Mediterranean sea, and is in agreement with several studies in different locations in the Mediterranean sea.

4.1. Introduction

The impact of invasive species on terrestrial and marine ecosystems is increasing (Kolar and Lodge, 2001). However, these phenomena also provide a chance to observe the process of evolution of the invaders in new habitats (Lockwood *et al.*, 2007). This is particularly important for the invaders that belong to marine environments (Lee, 2002; Wares *et al.*, 2005). A lot of efforts have been made to understand how these invasive species affect the ecosystems, and resources have been allocated to prevent and control the invasive species.

The field of phylogeography encompass the study of the processes responsible for the spatial distributions of individuals during evolutionary time. This is accomplished by linking genetic lineages and geographic distributions of individuals (Avise, 2000). Thus, phylogeography has been successfully used to identify invasive species and invasive potential of new colonists, in addition to the assessment of the succes of the invasion process (Peterson 2003). The approaches used to observe and assess the invasive species now include several genetic tools, which have proven effective to address several questions, such as the source and the timing of invasion (Byers and Goldwasser, 2001), the invasive patterns (Tsutsui *et al.*, 2000), the historical biogeography (Magoulas *et al.*, 1996), and the propensity of certain species to become successful invaders (Williamson and Fitter, 1996).

Invasive species are observed to be exposed to selective pressures, as they try to adapt to a new habitat. Pressure is typically posed by native species that compete with the invaders for food resources and habitats. The genetic attributes of invasive species have to determine how well the invaders can adapt and settle in the new habitat. The genetic characteristics of invasive species that are to be considered include epistasis, and genetic variance and trade-offs (Lee, 2002). Studying the genetic variance and observing the potential for evolution in these species can provide useful information about their invasive nature. Researching about their life histories may also prove to be helpful in deciding which of these species are likely to transform into harmful pests.

However, a first step to obtain information about the evolutionary processes of invasive species, especially as very little data is available for most invading species worldwide, is to describe the genetic structure of these taxa, in space and time.

4.2. The genetic of lessepsian bioinvasion

Bioinvasions can help provide an opportunity to research about the evolutionary processes in invasive species. These species can also help to identify various factors that support

invasions and the conditions needed for these organisms to adapt. Several intrinsic properties of invasive species, such as genetic variance, growth rate and tolerability to abiotic aspects, can influence the success rate of their invasion (Ehrlich, 1989; Byers and Goldwasser, 2001). The process of invasion is also said to be affected by several external factors such as competitors, predators and the presence of parasites (Mitchell and Power, 2003; Torchin *et al.,* 2002, 2003; Colautti *et al.,* 2004). For these organisms, new environments are usually quite different as compared to their native habitats in terms of ecological conditions. They may have varying levels of physiological stresses like salinity and temperature and may force the invading species to adapt. This adaptation takes place by the virtue of genetic variability (Golani, 1998; Tsutsui *et al.,* 2000). Managing the survival of these species in new habitats has been a controversial topic. More research on these invasive species introduced to new environments is needed, however, it is not known whether this research will help in increasing knowledge about their control and prevention.

Invasion of an environment by a new species takes time and often involves different stages, beginning from colonization and gradually proceeding to the expansion of population into the new habitats (Kowarik, 1995). It has been claimed by Holt *et al.,* (2005) that this time period is variable and may have strong impacts on the ecology and demography of the environment. It can be estimated by determining the duration required for the species to adapt to a new habitat. Genetic research can be useful in tracking the mechanism of colonisation in these species and can help in relating their genetic structure with their capacity of reacting to the process of natural selection (Lee, 2002; Allendorf and Lundquist, 2003). Understanding the mechanism of invasion in a marine ecosystem is difficult, especially when recruitment process is variable, cryptic species are present and the environment is not directly observable (Hastings *et al.,* 2005; Puth and Post, 2005; Wonham *et al.,* 2000).

The genetic features of native and invasive species have been contrasted using molecular techniques (Golani and Ritte, 1999; Bucciarelli *et al.,* 2002; Karako *et al.,* 2002; Bonhomme *et al.,* 2003; Hassan *et al.,* 2003; Azzurro *et al.,* 2006). In case of invasion by Lessepsian species, the procedure has been used to monitor their genetic structuring (Karako *et al.,* 2002; Azzurro *et al.,* 2006; Terranova *et al.,* 2006). The genetic study of lessepsian invasion have been also used to unveiled demographic aspects and population dynomics within the Mediterranean Sea (Azzurro *et al.,* 2006; Iannotta *et al.,* 2007). It has also clarified the classification of species (Golani and Ritte, 1999; Kasapidis *et al.,* 2007) and has helped to uncover several cryptic species (Bucciarelli *et al.,* 2002).

The Lessepsian invasion is relatively new and is was long believed that only a small number of organisms originating from the Red Sea had made their way to settle in the Mediterranean Sea. However, a lot of other species have just started their process of invading the Mediterranean Sea and many have already settled and passing through the process of expansion. Based on the updated checklists by Zenetos *et al.* (2010, 2011, 2012), a total of 986 invasive species are recorded in the Mediterranean Sea: 775 in the Eastern, 308 in the Western, 249 in the Central Mediterranean Sea and 190 in the Adriatic Sea. While, Shakman *et al.*, (2016) reported that the 35 marine invasive species recorded in the Libyan waters represent the highest percentage of invasive fish (71%) followed by molluscs (17.14%)and crustaceans (11.43 %).Molecular techniques still pose as an important procedure for studying biological invasions, concentrating on the stage of colonisation and taking samples of the species for studying their genetics. Golani *et al.* (2007) has suggested studying the small populations residing in the western edge whose invasions are still in the initial stages (Azzurro, 2006; Terranova *et al.*, 2006). Observing a population that has settled early is important for simplification of theoretical work and determination of basic variables in the process of colonisation (Lockwood *et al.*, 2007).Receiving information about the genetic structures of these species can help to understand various aspects such as the dispersal capability, reproductive features, connectivity and the biogeography (Shulman 1998; Palumbi 2003). For this reason, studies involving the genetic features of invasive species are becoming popular. Azzurro *et al.*, (2006) have contributed important knowledge on the Siganidae family, particularly the Lessepsian species such as *S. rivulatus and S. luridus*. These two species have been regarded as the most common and successful among the other 59 fish migrants belonging to Lessepsian category ever since 1869 when the Suez Canal was first opened. Azzurro *et al.* (2006) concluded that the *Siganus* species maintained the original mitochondrial diversity and there was no pattern of regional separation during the colonization of the island of Linosa, Italy. Such Information agrees with the thought that Lessepsian species includes a large number of individuals from the earliest stages of colonization. The size of propagule and the level of diversity in the founding individuals is key to the success of the invader, but multiple invasions can help dampen the genetic bottlenecks occurring at initial stages, with high levels of gene flow providing the invaders with added genetic diversity (Dlugosh and Parker, 2007).

4.3. Aims

The present genetic study aims at comparing the distribution of genetic diversity in *S. luridus* and *S. rivulatus* of newly sequenced data and in relation to previous data obtained by

other researchers in the previous decades. By pooling newly generated mitochondrial DNA sequences with previous data available from the literature, we attempt to relate genetic diversity in the two species with their known stage of colonisation on distributional spread.

4.4. Materials and methods

4.4.1. Collections samples

Samples were collected at four sites in the Cyrenaica coast - Libya and two sites in the Red sea. The samples were gathered in October and November 2014 and August and September 2015 directly from local anglers on the coast, as well as fishing boats that fish in the study areas, up to 20 m deep according to the study area and fishing licenses. About 25 samples have been collected for every species in each area, for a total of 521 specimens (375 specimens from Libya and 146 specimens from the Red Sea; Table 1). After collection, tissue samples were immediately placed in 95% ethanol and stored at ambient temperature in the field, and then at 4 °C in the lab.

4.4.2. DNA extraction, amplification and sequencing

Although the use of nuclear DNA is more suited for fine scale analysis of population structure, mitochondrial DNA (mtDNA) is more commonly usedfor recovering broad phylogeographic patters and gaining an overall view of recent patterns of spatial population expansions. In addition, mtDNA can be obtained from more degraded sources. In this study DNA was extracted using a Chelex protocol. An approximately 350 base pair (bp) fragment of the mitochondrial control region was accomplished using the primers CR-A ‘‘TTCCACCTCTAACTCCCAAAGCTAG’’ and CR-E ‘CCTGAAGTAGGAACCAGATG’’ (Lee *et al.,* 1995). All amplifications (20 ul) contained 0.2 µl of Taq, 2 µl buffer, 0.2 µl dNTPs, 0.6 µl MgCl2, 1 µl of each primer, and a fragment of approximately 350 bp of the gene cytochrome *b* was amplified by PCR using universal primers. PCR was carried out with an initial denaturation at 95°C for 5 min, followed by 35 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 52°C for 40 sec, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min). Automated sequencing was performed in both directions with the primers used in the amplification using an ABI 310 automated sequencer (Applied Biosystems, Foster City, California) PCR products were purified and sequenced by Source BioScience Life Sciences (http://www.lifesciences.sourcebioscience.com/).

4.4.3. Alignments, datasets, genetic analyses

Together with the control region sequences generated in this study for the Cyranaican populations in the Libyan Sea, The sequences of the control region for *S. luridus* and *S. rivulatus* from the Cyrenaica coast in the Libyan Sea were generated in the lab at Salford University.In addition, ninety-six sequences for *S. luridus* and seventy sequences for *S. rivulatus* were obtained from GenBank for other areas in the Mediterranean Sea (Italy, Greece, Turkey, and Israel) and from the Red sea (Eilat gulf) Shakman *et al.,* 2008 and Azzurro *et al*.,2005 (Table 13, 16).The sequences were visually controlled and finally aligned using the BioEdit software (Hall, 1999).

Population diversity indices such as numbers of segregating sites (S), haplotypes number (h), haplotype diversity (Hd) and nucleotide diversity (π) and the average number of pairwise nucleotide differences within the population (K), were calculated using DnaSP v 5.10 (Librado and Rozas, 2009). The pairwise genetic difference between the population was estimated for all populations by calculating Wright's F-statistics (F_{ST}) by population genetics package ARLEQUIN (Excoffier and Lischer, 2010) with the significance of pairwise differences at the level of 0.05 assessed with 10,000 permutations. Haplotype genealogies were constructed in the program HapView, following a method described by Salzburger *et al.* (2011).

The relationship among all samples of *Siganus* species collections was visualized by multi-dimensional scaling (MDS) of pairwise Φ_{ST} using the VEGAN package in R (Excoffier and Lischer, 2010). I estimated pair-wise genetic differentiation between populations obtained from ARLEQUIN, which includes information for haplotype frequencies. Pairwise Φ_{ST} were further linearized following as implemented in ARLEQUIN and the paired genetic distances between populations (matrix) were visualized using metric multidimensional scaling (MDS) performed with metaMDS in the vegan package in R. Differences and similarities in haplotype frequency between samples in both *Siganus* species were visualized using multidimensional analysis (MDS).

4.5. Results

4.5.1. Diversity indices of S. luridus population

In the present study, 138 Mitochondrial DNA sequence data of *S. luridus* were examined from 11 locations from the Mediterranean Sea produced a 383 bp fragment. Among these, a total of 84 haplotypes were found: 10 in West of Libya (LIB_W), 4 in Turkey (TUR_KAS), 8

in Italy (ITA_LIN), 5 in Greece (GRC_PAR), 5 in Greece (GRC_SIF), 9 in Israel (ISR_HAI), 16 in Israel (ISR_EIL) also, found in the 27 haplotypes in Cyrenaica population distributed as follows: 5 in Ain Al-gazal (CYR_AINL), 10 in Susah (CYR_SUL), 6 in Al-Haniya (CYR_HNL) and 6 in Tubruq(CYR_TBL) (Table 13). Haplotype diversity (Hd) for all the 138 sequences was calculated to be 0.902 +/- 0.012 SD. The average number of nucleotide differences,(k) was found to be 2.71 and nucleotide diversity (π) was 0.006 +/- 0.0002 SD. Haplotype and nucleotide diversity indices were highest in West of Libya(LIB_W) population followed by Greece (GRC_PAR) population and lowest in in Greece (GRC_SIF) and in Cyrenaica population, Al-gazal (CYR_AINL). Haplotype diversity (Hd) of *S. luridus* was found to be higher in Libya population (LIB_W) and the Italy population (ITA_LIN; Hd=1.000) than in any Mediterranean populations. In the Cyrenaica coast, obvious trend was apparent, with the diversity of the Mediterranean Sea (Hd= 0.919) being similar to the diversity observed on the Red sea coast of Israel (Hd = 0.966), while, Haplotype diversity (Hd) for the Turkey population was low (Hd=0.75).

Nucleotide diversity (π) followed the same trend as Haplotype diversity (Hd), with a higher diversity in the Libya population (LIB_W) and the Italy population (ITA_LIN) (π = 0.01) than in the Red sea populations (π=0.007), and similar diversity levels within the Mediterranean (Table 13).

The average number of segregating sites (s) was lower in the all Mediterranean population novel habitats than in the Red sea population, native habitats (ISR_EIL) native populations (14) of *S.luridus* expect the Libyan population (LIB_W). The variance in the number of segregating sites of *S. luridus* population was higher in the Libya population (LIB_W) and the Israel population in the Red sea (ISR_EIL) (s = 15 and s = 14) respectively. While the number of segregating sites (s) were lows in the Cyrenaica population (s = 6,10,4 and 7) in the CYR_AINL;CYR_SUL;CYR_HNL and CYR_TBL respectively, the average number of pairwise nucleotide differences (k) followed the same trend with the Haplotype diversity (Hd) were found (4.51 and 4) in the Libyan population (LIB_W) and the Greece population (GRC_PAR)

Inter-population of *S. luridus* the average proportion of nucleotide differences between populations (Kxy) varied from 1.83 Turkey (TUR_KAS) and Italy (ITA_LIN) to 4.54 Libya (LIB_W) and Greece (GRC_PAR) respectively, and average number of nucleotide substitutions per site between all *S. luridus* populations (Dxy) varied from 0.004 Turkey (TUR_KAS) and Italy (ITA_LIN) also, Turkey (TUR_KAS) and Israel (ISR_HAI) to 0.01 Libya (LIB_W) and Greece (GRC_SIF) respectively (Table 14).

Significant F_{ST} and corrected pairwise differences were found between all Cyrenaica population and The Red sea populations also all Mediterranean population. Significant differences were also found between the Cyrenaica populations and some other adjacent locations in the Mediterranean Sea for example, Italy, Greece, Turkey and Israel (Table.15). The result of the MDS plot analysis showed differences between populations of *S. luridus* according to mtDNA (Fig.50). As displayed in the MDS plot, there was a grouping into Cyrenaica populations.The MDS plots for *S. rivulatus*, visualising similarity between populations in Cyrenaica and the Mediterranean population on mtDNA, although the *S. luridus* were separated into two clusters in the mtDNA data.

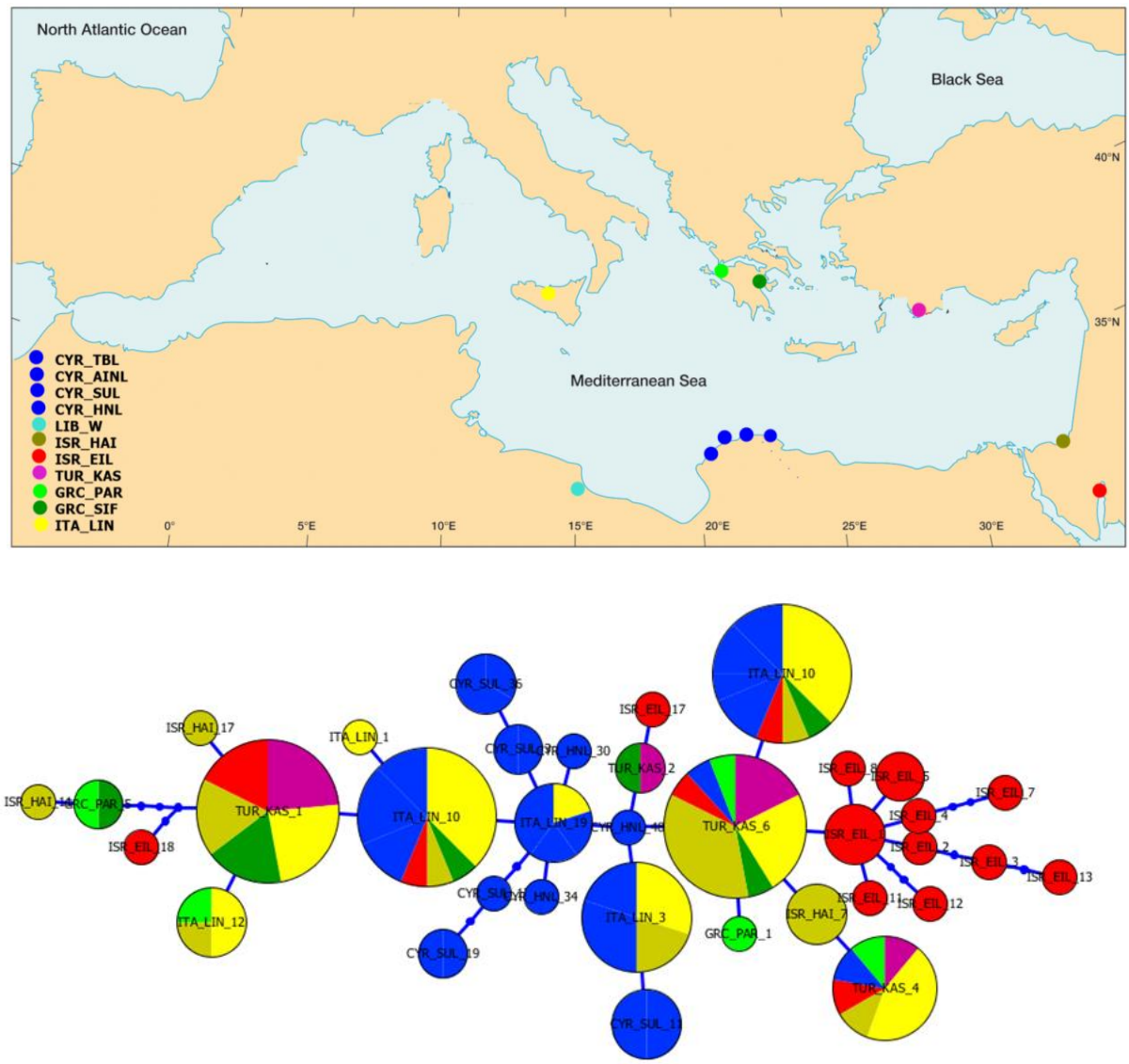


Figure 49. Top: Geopraphic map showing the location of the *Siganus luridus* samples collected for this study and obtained from GenBank. Bottom: Haplotype network of *S. luridus*. Colours refer to the location as shown in the top panel, with the exception of LIB-W, which was removed from the analysis (please see appendix 7.1).

Table 13. Control region sequences for Siganus luridus. N: Number of sequences examined for each location (Code abbreaviated as in Fig. 49); S: Number of segregating sites; Hn: Number of haplotypes; Hd: Haplotype diversity; k: Average number of pairwise nucleotide differences; π: nucleotide diversity.

Code	Locations	N	S	Hn	Hd	k	π	Date	Reference
LIB_W	Libya-west	10	15	10	1	4.51	0.011	2007	Shakman *et al.*, 2008
TUR_KAS	Turkey	9	5	4	0.75	1.77	0.009	2000	Azzurro *et al.*,2005
ITA_LIN	Italy	25	6	8	0.876	2	0.005	2000	Azzurro *et al.*,2005
GRC_PAR	Greece	5	9	5	1	4	0.01	2000	Azzurro *et al.*,2005
GRC_SIF	Greece	7	7	5	0.857	2.66	0.006	2000	Azzurro *et al.*,2005
ISR_HAI	Israel	19	11	9	0.871	2.2	0.005	2000	Azzurro *et al.*,2005
ISR_EIL	Israel	21	14	16	0.966	3.01	0.007	2000	Azzurro *et al.*,2005
CYR_AINL	Libya_Cyrenaica	8	6	5	0.857	2.39	0.006	2014	This study
CYR_SUL	Libya_Cyrenaica	18	10	10	0.901	2.71	0.007	2014	This study
CYR_HNL	Libya_Cyrenaica	8	4	6	0.928	1.64	0.004	2014	This study
CYR_TBL	Libya_Cyrenaica	8	7	6	0.928	2.92	0.006	2014	This study
		Total (138)	**Total (94)**	**Total (84)**	**Average (0.902)**	**Average (2.71)**	**Average (0.006)**		

Table 14. Population genetics indices between different populations of *S. luridus* calculated from sequence of mitochondrial DNA.

Population 1	Population 2	K_{xy}	D_{xy}	D_a	G_{ST}
LIB_W	TUR_KAS	3.410	0.008	0.001	0.066
LIB_W	ITA_LIN	3.500	0.009	0.001	0.033
LIB_W	GRC_PAR	4.540	0.011	0.001	0.008
LIB_W	GRC_SIF	3.840	0.010	0.001	0.037
LIB_W	ISR_HAI	3.680	0.009	0.001	0.034
LIB_W	ISR_EIL	4.470	0.011	0.002	0.012
LIB_W	CYR_AINL	3.770	0.009	0.001	0.037
LIB_W	CYR_SUL	4.080	0.010	0.001	0.026
LIB_W	CYR_HNL	3.550	0.009	0.001	0.019
LIB_W	CYR_TBL	4.150	0.010	0.002	0.019
TUR_KAS	ITA_LIN	1.830	0.004	0.000	0.028
TUR_KAS	GRC_PAR	2.620	0.006	-0.001	0.025
TUR_KAS	GRC_SIF	2.100	0.005	0.000	-0.036
TUR_KAS	ISR_HAI	1.870	0.004	0.000	0.009
TUR_KAS	ISR_EIL	2.700	0.007	0.001	0.032
TUR_KAS	CYR_AINL	2.340	0.006	0.001	0.102
TUR_KAS	CYR_SUL	2.740	0.007	0.001	0.084
TUR_KAS	CYR_HNL	2.250	0.005	0.001	0.088
TUR_KAS	CYR_TBL	2.610	0.006	0.001	0.067
ITA_LIN	GRC_PAR	2.880	0.007	0.000	0.021
ITA_LIN	GRC_SIF	2.360	0.006	0.000	0.017
ITA_LIN	ISR_HAI	2.090	0.005	0.000	0.007
ITA_LIN	ISR_EIL	2.890	0.007	0.001	0.016
ITA_LIN	CYR_AINL	2.320	0.006	0.000	0.012
ITA_LIN	CYR_SUL	2.670	0.006	0.001	0.014
ITA_LIN	CYR_HNL	2.170	0.005	0.001	0.022
ITA_LIN	CYR_TBL	2.570	0.006	0.001	0.018
GRC_PAR	GRC_SIF	3.000	0.007	-0.001	0.010
GRC_PAR	ISR_HAI	2.850	0.007	-0.001	0.014
GRC_PAR	ISR_EIL	3.700	0.009	0.001	0.022
GRC_PAR	CYR_AINL	3.520	0.009	0.001	0.028
GRC_PAR	CYR_SUL	3.960	0.010	0.002	0.033
GRC_PAR	CYR_HNL	3.550	0.009	0.002	0.022
GRC_PAR	CYR_TBL	3.800	0.010	0.002	0.010
GRC_SIF	ISR_HAI	2.410	0.006	0.000	0.017
GRC_SIF	ISR_EIL	3.140	0.008	0.001	0.017
GRC_SIF	CYR_AINL	2.940	0.008	0.001	0.059
GRC_SIF	CYR_SUL	3.260	0.009	0.001	0.051
GRC_SIF	CYR_HNL	2.640	0.007	0.001	0.038
GRC_SIF	CYR_TBL	3.000	0.008	0.001	0.029
ISR_HAI	ISR_EIL	2.950	0.008	0.001	0.020
ISR_HAI	CYR_AINL	2.500	0.007	0.001	0.042
ISR_HAI	CYR_SUL	2.900	0.008	0.001	0.039
ISR_HAI	CYR_HNL	2.480	0.006	0.001	0.046
ISR_HAI	CYR_TBL	2.840	0.007	0.001	0.029
ISR_EIL	CYR_AINL	3.260	0.009	0.001	0.037
ISR_EIL	CYR_SUL	3.600	0.009	0.002	0.029
ISR_EIL	CYR_HNL	3.090	0.008	0.002	0.025
ISR_EIL	CYR_TBL	3.450	0.009	0.002	0.023
CYR_AINL	CYR_SUL	2.410	0.006	0.000	-0.019
CYR_AINL	CYR_HNL	2.000	0.005	0.000	0.007
CYR_AINL	CYR_TBL	2.710	0.007	0.001	0.024
CYR_SUL	CYR_HNL	2.160	0.006	0.000	0.007
CYR_SUL	CYR_TBL	2.690	0.007	0.000	0.007
CYR_HNL	CYR_TBL	2.250	0.006	0.001	-0.004

K_{xy}: Average proportion of nucleotide differences; D_{xy}: average number of nucleotide substitutions per site; D_a: number of net nucleotide substitutions per site; G_{ST}: Genetic differentiation index based on the frequency of haplotype

Table 15. Pairwise population genetic differentiation between populations of *S. luridus*, based on mtDNA. Φ_{ST} below diagonal, relative p-values above diagonal. Significant values in **bold** ($p<0.05$) or **bold*** ($p<0.01$).

Population	LIB_W	TUR_KAS	ITA_LIN	GRC_PAR	GRC_SIF	ISR_HAI	ISR_EIL	CYR_AINL	CYR_SUL	CYR_HNL	CYR_TBL
LIB_W		0.054	0.009	0.216	0.09	0	0	0.099	0	0.018	0.009
TUR_KAS	0.072		0.747	0.801	0.576	0.99	0.027	0.135	0	0	0
ITA_LIN	**0.116***	-0.035		0.351	0.234	0.45	0	0.081	0.009	0.018	0.009
GRC_PAR	0.055	-0.067	0.019		0.81	0.648	0.063	0.09	0.018	0	0
GRC_SIF	0.053	-0.046	0.028	-0.10		0.423	0.045	0.063	0.009	0	0.018
ISR_HAI	**0.114***	-0.065	-0.004	-0.046	-0.018		0	0.045	0	0.009	0
ISR_EIL	**0.176***	**0.090**	**0.136***	0.079	**0.087**	**0.115***		0	0	0	0
CYR_AINL	0.076	0.114	0.065	0.116	0.142	**0.084**	**0.158***		0.882	0.594	0.054
CYR_SUL	**0.134***	**0.164***	**0.124***	**0.193**	**0.174***	**0.153***	**0.206***	-0.059		0.549	0.153
CYR_HNL	**0.117**	**0.248***	**0.147**	**0.248***	**0.190***	**0.209***	**0.210***	-0.008	-0.021		0.126
CYR_TBL	**0.153***	**0.197***	**0.151***	**0.178***	**0.150**	**0.190***	**0.197***	0.113	0.041	0.095	

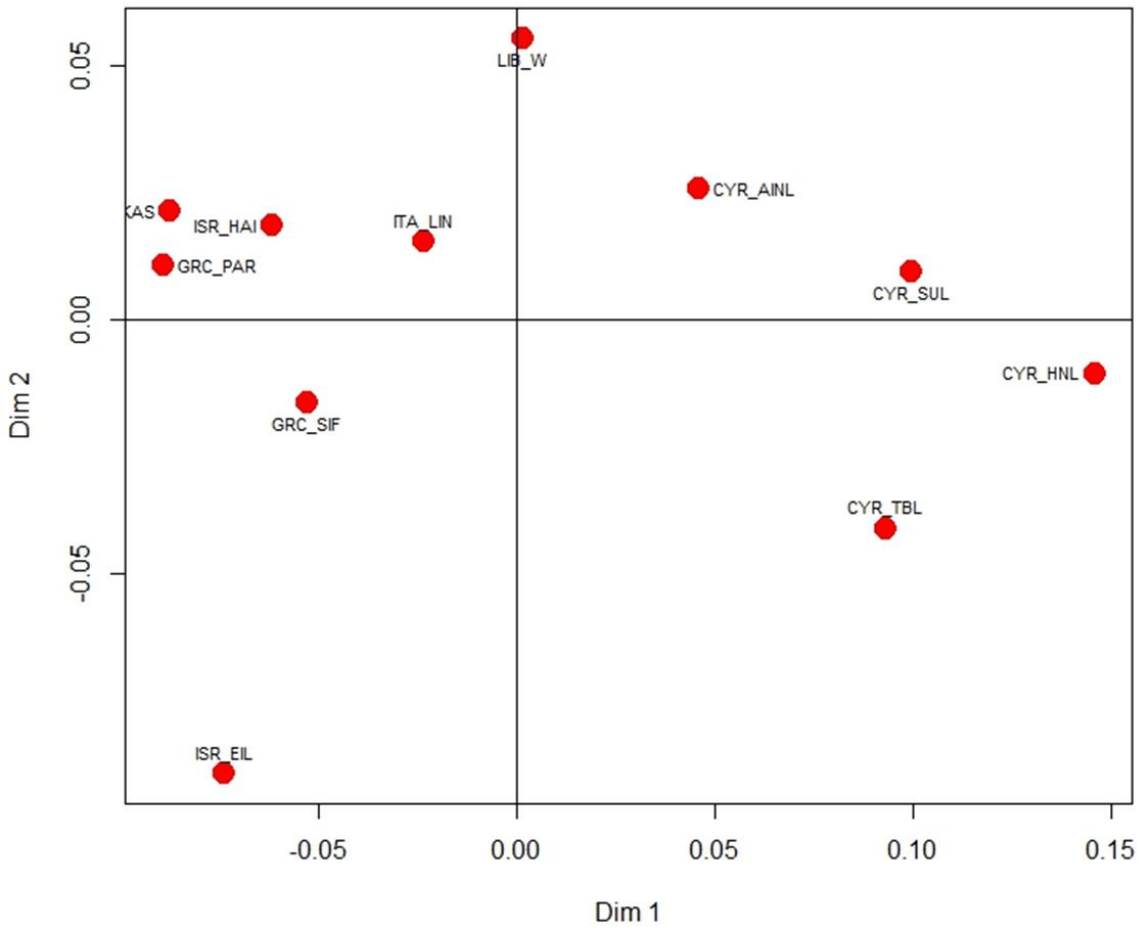


Figure 50. Multidimensional Scaling Plot (MDS) of *S. luridus* mtDNA differentiation from 11 collections Φ_{ST} pairwise genetic distances across the Mediterranean Sea (Location codes see Table 13).

4.5.2. Diversity indices of *S. rivulatus* population

Analyses of the Mitochondrial DNA produced a fragment of 383 bp of *S. rivulatus.* Thereby, the 107 sequences analysed resulted in 78 haplotypes, 22 of which are exclusive for the Cyrenaica populations and 52 exclusive for the Mediterranean Sea and 4 for Rd Sea.

Haplotype diversity (Hd) was found to be lower in Cyrenaica populations (CYR_TBR; CYR_SUR; CYR_AINR and CYR_HNR) Average (Hd = 0.759) than the Red Sea populations (Hd = 1.000), as well as in the combined west Libyan populations (LIB-W) the haplotype diversity (Hd = 1.000) was higher than Cyrenaica populations. Nucleotide diversity followed the same trend, with a lower diversity in the Cyrenaica populations (π= 0.006) than in the Red sea populations (π= 0.009), and similar diversity levels within the Mediterranean (Table 16).

The average number of segregating sites (s) was higher in the all Libyan population (LIB_W) novel habitats (s = 38) than in the Red sea population, native habitats (ISR_EIL) native populations (s = 6) of *S. rivulatus* expect the Cyrenaica population (CYR_TBR and CYR_HNR) were (s = 14 and 11) respectively. The average number of pairwise nucleotide differences (k) followed the same trend with the average number of segregating sites (s) were found (5.59 and 3.82) in the Libyan population (LIB_W) and the Cyrenaica population (CYR_HNR)

Inter-population of *S. rivulatus* the average proportion of nucleotide differences between populations (Kxy) varied from 1.00 Turkey (TUR_KAS) and Cyrenaica (CYR_SUR) to 5.17 Libya (LIB_W) and Israel (ISR_EIL) respectively, and average number of nucleotide substitutions per site between all *S. rivulatus* populations (Dxy) varied from 0.002 Turkey (TUR_KAS) and Cyrenaica (CYR_SUR) to 0.013 Libya (LIB_W) and Israel (ISR_EIL) (Table 17).

Significant F_{ST} and corrected pairwise differences were found between all Cyrenaica population and The Red sea populations also all Mediterranean population. Significant differences were also found between the Cyrenaica populations and some other adjacent locations in the Mediterranean Sea for example, Italy, Greece, Turkey and Israel (Table.18).

The F_{ST} values were not significantly different between Cyrenaica populations and the Red sea populations (Israel, ISR_EIL) of *S. rivulatus* except, the Cyrenaica population from Al-haniya (CYR_HNR). While, the F_{ST} values were significantly

different between Cyrenaica populations form the Susah (CYR-SUR) with the population of Israel (ISR_EIL), also between the population from the Cyrenaica (CYR_AINR) with the populations from Israel and Turkey in the Mediterranean Sea (TUR_KAS).

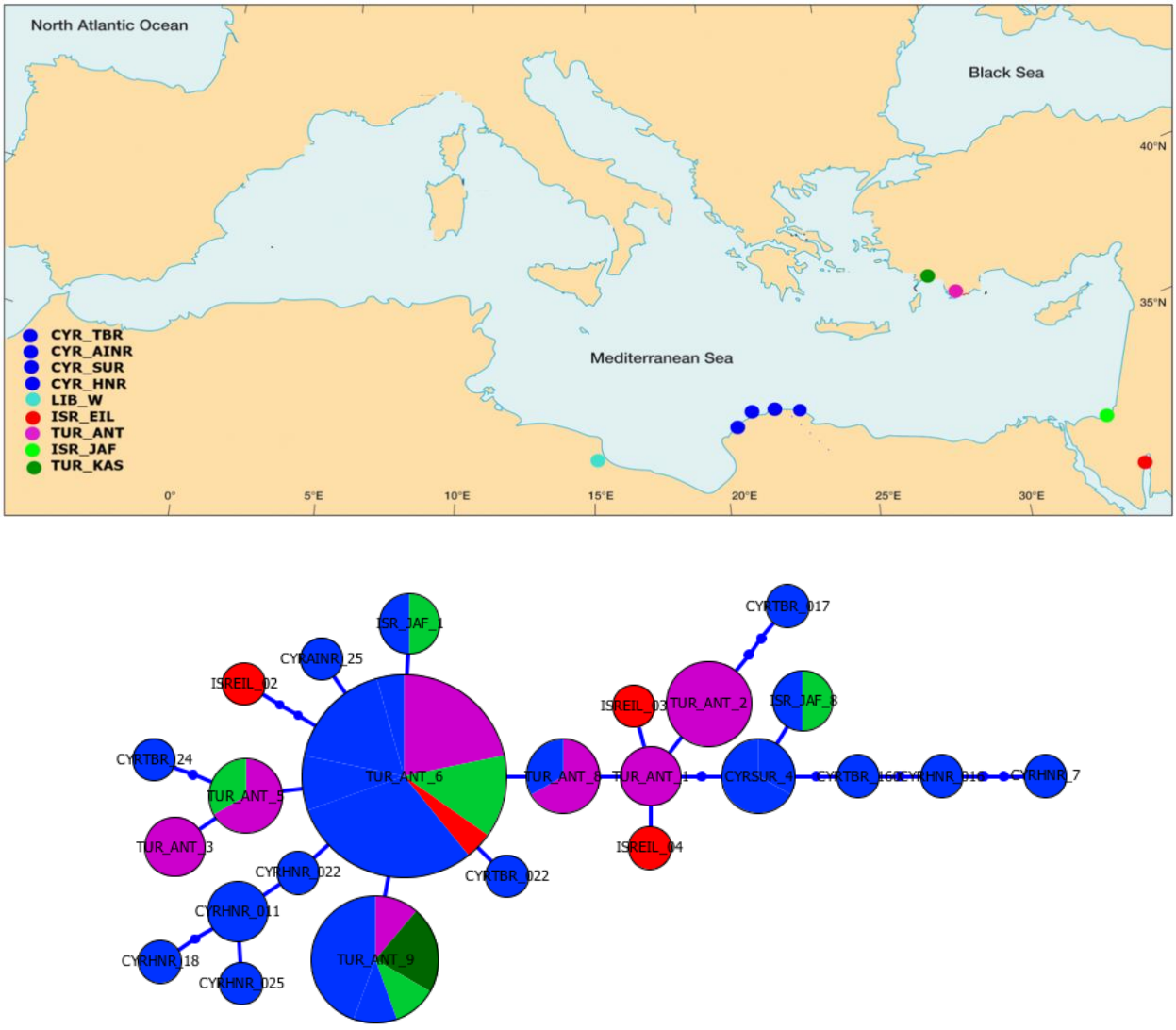


Figure 51. Top: Geopraphic map showing the location of the *Siganus rivulatus* samples collected for this study and obtained from GenBank. Bottom: Haplotype network of *S. rivulatus*. Colours refer to the location as shown in the top panel, with the exception of LIB-W, which was removed from the analysis (please see appendix 7.2).

Table 16. Control region sequences for *Siganus rivulatus*. N: Number of sequences examined for each location (Code abbreaviated as in Fig. 51); S: Number of segregating sites; Hn: Number of haplotypes; Hd: Haplotype diversity; k: Average number of pairwise nucleotide differences; π: nucleotide diversity

Code	Locations	N	S	Hn	Hd	k	π	Date	Reference
LIB_W	Libya -west	39	38	39	1	5.59	0.014	2007	Shakman *et al.*, 2008
TUR_ANT	Turkey	18	6	7	0.869	2.04	0.005	2000	Azzurro *et al.*,2005
TUR_KAS	Turkey	2	0	1	0	0	0	2000	Azzurro *et al.*,2005
ISR_JAF	Israel	7	8	5	0.857	2.286	0.005	2000	Azzurro *et al.*,2005
ISR_EIL	Israel	4	6	4	1	3.5	0.009	2000	Azzurro *et al.*,2005
CYR_TBR	Libya _ Cyrenaica	15	14	9	0.8	2.89	0.007	2014	This study
CYR_SUR	Libya_Cyrenaica	7	5	3	0.666	1.71	0.004	2014	This study
CYR_AINR	Libya_Cyrenaica	7	5	3	0.666	2.19	0.005	2014	This study
CYR_HNR	Libya_Cyrenaica	8	11	7	0.904	3.82	0.009	2014	This study
		Total (107)	**Total (93)**	**Total (78)**	**Average (0.677)**	**Average (2.66)**	**Average (0.006)**		

Table 17. Population genetics indices between different populations of *S. rivulatus* calculated from sequence of mitochondrial DNA.

Population 1	Population 2	Kxy	Dxy	Da	GST
LIB_W	TUR_ANT	4.206	0.01	0.001	0.031
LIB_W	TUR_KAS	4.205	0.01	0.003	0.13
LIB_W	ISR_JAF	4.183	0.01	0.006	0.037
LIB_W	ISR_EIL	5.173	0.013	0.001	0.043
LIB_W	CYR_TBR	4.5	0.011	0.006	0.044
LIB_W	CYR_SUR	4.219	0.011	0.001	0.061
LIB_W	CYR_AINR	4.256	0.011	0.009	0.061
LIB_W	CYR_HNR	6.31	0.016	0.004	0.021
TUR_ANT	TUR_KAS	2.277	0.005	0.003	0.17
TUR_ANT	ISR_JAF	2.23	0.005	0.001	0.006
TUR_ANT	ISR_EIL	2.861	0.007	0.002	0.028
TUR_ANT	CYR_TBR	2.492	0.006	0.006	0.014
TUR_ANT	CYR_SUR	2.246	0.005	0.009	0.065
TUR_ANT	CYR_AINR	2.23	0.005	0.002	0.043
TUR_ANT	CYR_HNR	4.305	0.011	0.003	0.028
TUR_KAS	ISR_JAF	1.857	0.004	0.001	0.213
TUR_KAS	ISR_EIL	3.25	0.008	0.003	0.261
TUR_KAS	CYR_TBR	2.466	0.006	0.002	0.193
TUR_KAS	CYR_SUR	1	0.002	0.003	0.101
TUR_KAS	CYR_AINR	2.285	0.005	0.003	0.33
TUR_KAS	CYR_HNR	4.125	0.01	0.005	0.213
ISR_JAF	ISR_EIL	3.107	0.008	0.056	-0.011
ISR_JAF	CYR_TBR	2.4	0.006	-0.005	-0.023
ISR_JAF	CYR_SUR	1.95	0.005	-0.001	0.021
ISR_JAF	CYR_AINR	2.102	0.00549	-0.003	-0.004
ISR_JAF	CYR_HNR	4.16	0.01	0.002	0.019
ISR_EIL	CYR_TBR	3.383	0.008	0.004	0.019
ISR_EIL	CYR_SUR	3.107	0.008	0.001	0.059
ISR_EIL	CYR_AINR	2.964	0.007	0.003	0.02
ISR_EIL	CYR_HNR	5.125	0.013	0.003	0.004
CYR_TBR	CYR_SUR	2.438	0.006	0.0003	0.057
CYR_TBR	CYR_AINR	2.428	0.006	-0.003	0.006
CYR_TBR	CYR_HNR	4.475	0.011	0.002	0.034
CYR_SUR	CYR_AINR	2.102	0.005	0.003	0.088
CYR_SUR	CYR_HNR	4.089	0.01	0.003	0.082
CYR_AINR	CYR_HNR	4.08	0.01	0.002	0.064

K_{xy}: Average proportion of nucleotide differences; D_{xy}: average number of nucleotide substitutions per site; D_a: number of net nucleotide substitutions per site; G_{ST}: Genetic differentiation index based on the frequency of haplotype

Table 18. Pairwise population genetic differentiation between populations of *S. rivulatus*, based on mtDNA. Φ_{ST} below diagonal, relative p-values above diagonal. Significant values in **bold** ($p<0.05$) or **bold*** ($p<0.01$).

Population	LIB_w	TUR_ANT	TUR_KAS	ISR_JAF	ISR_EIL	CYR_TBR	CYR_SUR	CYR_AINR	CYR_HNR
LIB_w		0	0.459	0.234	0.081	0.063	0.018	0.135	0
TUR_ANT	**0.068***		0.099	0.189	0.135	0.243	0.081	0.144	0
TUR_KAS	0.007	0.286		0.252	0.243	0.135	0.684	0.054	0.036
ISR_JAF	0.008	0.034	0.112		0.027	0.99	0.531	0.99	0.009
ISR_EIL	0.065	0.094	0.26	**0.096**		0.135	0.054	0.333	0.018
CYR_TBR	0.038	0.01	0.114	-0.086	0.075		0.207	0.819	0
CYR_SUR	**0.061**	0.151	-0.163	-0.02	0.207	0.032		0.153	0
CYR_AINR	0.029	0.053	0.283	-0.064	0.069	-0.056	0.071		0.009
CYR_HNR	**0.221***	**0.359***	**0.296**	**0.259***	**0.279**	**0.263***	**0.313***	**0.25***	

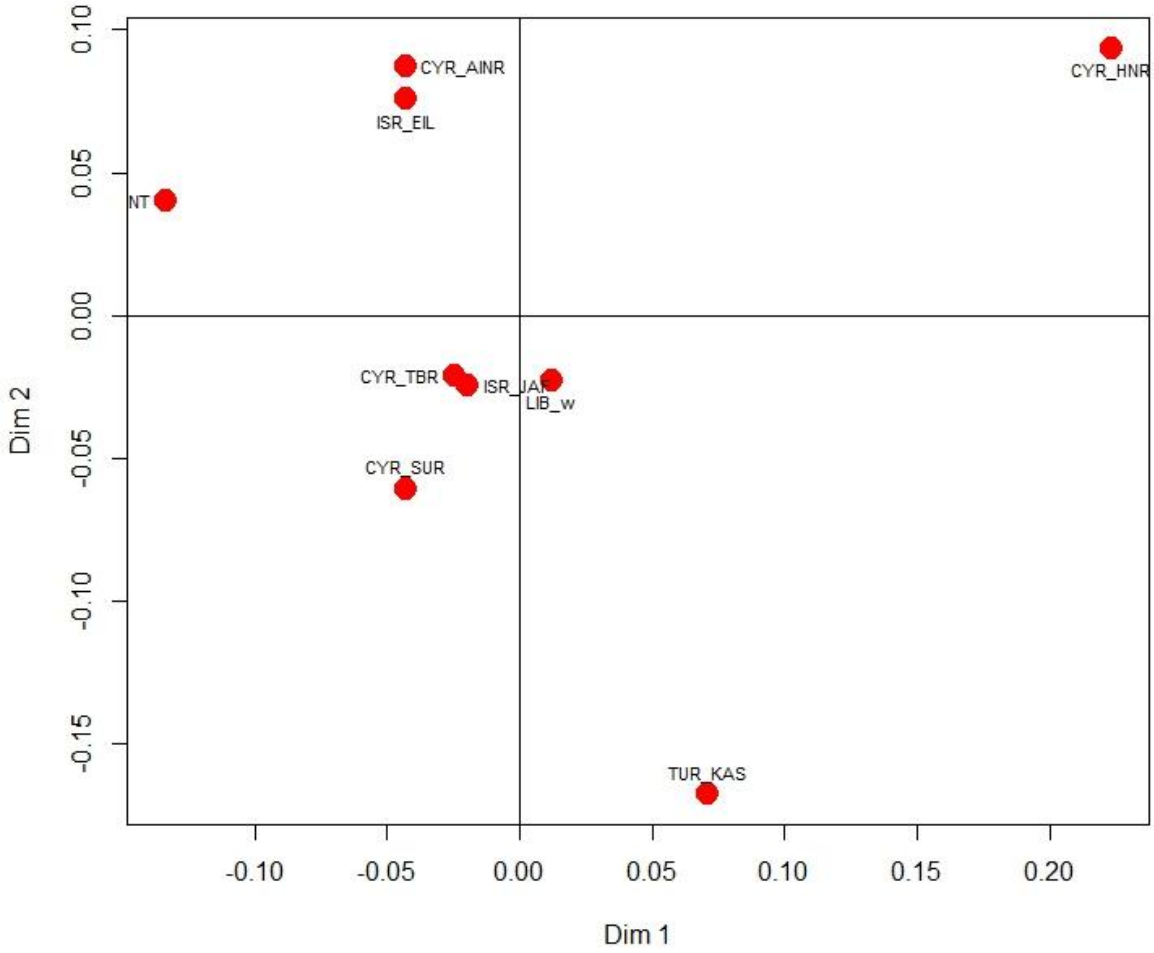


Figure 52. Multidimensional Scaling Plot (MDS) of *S. rivulatus* mtDNA differentiation from 9 collections Φ_{ST} pairwise genetic distances across the Mediterranean Sea (Location codes: see Table 16).

4.6. Discussion

In the present study, it was examined mtDNA of *S. luridus* in 11 samples and *S. rivulatus* in 9 samples from the Red Sea and Mediterranean Sea. The results have shown based in the mtDNA data (383bp) higher haplotype diversity (Hd) (0.966) and higher nucleotide diversity (π), (0.07) in the Isreal populations in the Red sea (ISR_EIL) than the Cyrenaica population and any other population except the Greece population (GRC_PAR). While the results of *S. rivulatus* were quite similar, the Haplotype diversity (Hd) were lower in the Cyrenaica population than the Red sea population, as well as the Nucleotide diversity (π) followed the same trend, with a lows diversity in the Cyrenaica populations than the Red sea population, this result is quite similar to the result that was obtained by Shakman *et al.*, (2008) and Hassan *et al.,* (2003).

According to Grant and Bowen (1998) and Tzeng (2007) that the marine fishes which have high haplotype diversity (Hd) and low nucleotide diversity (π) probably underwent population expansion in new habitats after a period of invasion. The data showed quite similar to haplotype diversity (Hd) between Cyrenaica populations and the Red sea populations of *S. luridus*, with a lower nucleotide diversity in the Cyrenaica population than the Red sea population.

Based on the haplotype networks using mtDNA of the *S. luridus* and *S. rivulatus* were well differentiated, did not separate into native population (Red sea) and Mediterranean population, may reason for the high levels of gene flow between the Red Sea and the Mediterranean sea, this result agrees with several studies in different locations in the Mediterranean sea (Hassan *et al.* 2003, Azzurro *et al.* 2006; Shakman *et al.,* 2008).

No significant difference of genetic differentiation was observed at mtDNA between the Cyrenaica population and the Red sea also with all population from the Mediterranean sea of *Siganus luridus*, this data agrees with many studies that have been done by Bonhomme *et al.,* (2003) concluded that, the *Siganus* species has been preserved the mitochondrial diversity during the colonisation process in the Mediterranean sea.

The analysis of *Siganus luridus* and *S. rivulatus* populations of Cyrenaica coast allowed to conclude that mitochondrial diversity is maintained during the colonisation process, as our data illustrate that the mitochondrial DNA diversity of both species have maintained intact their original mitochondrial diversity during colonisation of the Cyrenaica locations. Accordingly, to mitochondrial DNA diversity may reveal a wide ecophysiological plasticity in *S. rivulatus* and

S.luridus and may explain the success of these two *Siganus* species and adaptation in the novel habitats, according to Bariche (2002).

The previous studies by Golani *et al.* (2007) and Frankham (2005) proved that High migration rates, for the invasive species in the new habitats reduce the genetic diversity for them.Golani *et al.,* (2007) said that the invasion success and adaptation in the new habitats, it's difficult to attribute for the Genetic diversity.

This data confirms the hypothesis proposed by Dagit *et al.*(2007) suggesting that *S. luridus* and *S. rivulatus* within the Mediterranean Sea represents a no segregated population from the Red Sea. This data with information accorded with the idea that the both *Siganus* species migration involves many individuals since its beginning, also are rather the product of a constant or repeated influx of individuals from the Red Sea to the Mediterranean. The absence genetic differentiation between Cyrenaica populations and Red sea populations in *S. luridus* and *S. rivulatus* showed that a great number of migrants participated in the invasion of the Cyrenaica coast.

Haplotype frequencies were not significantly different between *S. rivulatus* in the Cyrenaica populations and the Red and Mediterranean population as shown by the F_{ST} analysis, the pattern observed of population form Al-haniya (CYR_HNR) for rare haplotypes being due to sampling variance These results indicate that *S. rivulatus* and have maintained intact their original mitochondrial diversity during colonisation of the Cyrenaica habitats. On the other hand, the F_{ST} values were statistically significant for *Siganus luridus* ($p < 0.05$), between the four populations from Cyrenaica and all population from the Red Sea and the Mediterranean sea, suggesting a recent rapid demographic expansion. Shakman et al (2008) refers that the abundance of *Siganus* species varied in the different regions, while the *S. rivulatus* was abundant in the Cyrenaica coast of Libya, the *S. luridus* was more abundant in the Gulf of Sidra and the western part of Libyan coast. As mentioned by Shakman *et al.*, (2008) that the reason for differences abundants between the both *Siganus* species in the Libyan waters probably the *S. rivulatus* is euyecous and adapts well to most habitats, whilst the stenecous *S. luridus* was found on one specific habitat (rock with algae).

Despite the wide distributional range of *Siganus* species in different locations in the Mediterranean sea and the Red sea, the estimation of inter-population comparison (Kxy): Average proportion of nucleotide differences between *S. rivulatus* populations, (Dxy): The average number of nucleotide substitutions per site between *S. rivulatus* populations, (Da) : The number of net nucleotide substitutions per site between populations, (Gst) : Genetic differentiation index based

on the frequency of haplotype, confirms and support the absence of genetic differentiation in *S. luridus* and *S. rivulatus.*

The Cyrenaica population, Red sea populations, and the Mediterranean population are highly closely related to each other which were suggested by a very low and non-significant F_{ST} value.

In general, the pattern of haplotype diversity (Hd) found for the *Siganus* species in the Cyrenaica habitats follows that reported for other Lessepsian migration, with higher indices in the red sea population and lower in the Cyrenaica population. The Cyrenaica populations clearly stood out, with lower of haplotypes diversity (Hd). Similar results were described for other Lessepsian migration fish species like *Fistularia commersonii* by Golani *et al.*, (2007). Also this results of this study allowed to confirm the assumption continue of a gene flow in the *Siganus luridus* and *Siganus rivulatus* from the Red Sea to the Mediterranean, and suggest that continued influx of genetic variation from the native habitat can provide rabbitfishes with sufficient adaptive plasticity to become successful in the new habitat.

Regarding the timing of the invasion process for both species, it is possible to refer to records from the literature: the first record of *S. rivulatus* in the eastern Mediterranean dates back to 1927 (Steinttz, 1927) while the first record of *S. luridus* was in 1956 (Bentuvia, 1964). Both species were recorded in Libya in 1970 (Stirn, 1970). Even though *S. rivulatus* was recorded earlier than *S. luridus*, the two species do not apper to have different genetic variation compared to the original populations. Since mtDNA control region should be more sensitive to demographic events in novel habitats because of its reduced active size, the data indicate that the Cyrenaican populations of the *S. luridus* and *S. rivulatus* are the product of a constant influx of individuals from the Red Sea to the Mediterranean Sea. Similar results were also obtained by Bucciarelli *et al.,* (2002) using mtDNA of another Lessepsian fish species, *A. lacunosus*.

On the other hands, the data from the western Libyan coast (see Appendix 7.1 and 7.2) by Shakman *et al.* (2008) for both species appear suspicious, because all individuals have different sequences, which is highly unlikely, based on all other data set from different locations in the Mediterranean Sea and the use of such data can lead to erroneous interpretations and even illogical conclusions. According to Lal and Lal (2011), the sequence errors in GenBank can originate from two sources: either from the sequences deposited in the database or from errors in the annotations. Therefore valid sequence data will save time and effort for researchers and will in the future help in making bioinformatics study an even stronger field of genetic analysis. However, with the

increased flow of sequence data in the database, it is difficult to check and validate all data. , a concern which has been , where Shum *et al.,* (2016) raised issues concerning the accuracy of the submitted sequence data and pointed out that they might not be free from errors.

Chapter V

5. Final Discussion

The Mediterranean Sea is one of the world's hot spots for biological invasions, and the documentation rates of invasive species in the Mediterranean have increased during the last two decades. Especially, there are two main entrances or ports of the invasive species of the Mediterranean, one of them is the Strait of Gibraltar and the other is the Suez Canal. Over the coming decades, there may be a greater flow of invasive species through the Suez Canal, especially after the creation of a new canal in 2015 in addition to the previous canal, allowing the increase of the flow rate of invasive species to the eastern part of the Mediterranean.

A relatively large number of invasive marine species of various origins have been reported in the different areas in the Mediterranean Sea, but data available on these invasive species in whole the Mediterranean Sea remains incomplete. Increase the spread of lessepsian species from Red Sea to the eastern Mediterranean and their rapid spread westwards. Similarly, it will also assist the spread of species of tropical Atlantic origin into the eastern basin in the Mediterranean Sea. There is much data evidence that the species composition of the eastern and western Mediterranean fish fauna has changed. Ketchum,(1983) reported that the taxa of the Mediterranean sea consist of Atlantic origin (67%), migrants through the Suez canal represent 5% of the total.

Moreover, recent studies for atlantic invasive species reported that some species continuous dispersal throughout the whole western basin, for instance the opah *Lampris guttatus*, the ringneck blenny *Parablennius pilicornis* and the bastard grunt *Pomadasys incisus*), also some atlantic invasive species patchy dispersal, for example *Kyphosus sectatrix* and the snake eel *Pisodonophis semicinctus* Otero *et al.,(*2013).

Soto-Navarro *et al.,* (2012) pointed out that, the rising salinity in Atlantic waters, implying a higher salinity input into the Mediterranean. The sharp rise of Atlantic species in the western basin in the Mediterranean Sea since 2006 may be related to this trend.

It is known that these invasive species recorded in the Mediterranean belong to different species and type, including Algae and seaweed in addition to invertebrates as well as fish. Some of these invasive species have spread widely in the Mediterranean and have been able to form large communities in different regions and environments, leading to a radical change in the biodiversity of these areas. This resulted in the establishment of mixed marine communities between the coming species of the Red Sea and the original species of the Mediterranean in some areas,

especially in the eastern Mediterranean, which saw the recording of large numbers of invasive species.

The phenomenon of invasive species leads to the disruption of biodiversity patterns, by affecting native species through competition for food sources or through predation or transfer of some pests or diseases to the new environments. This can lead to the loss of some species and the alteration of community dynamics and ecosystem function and services Bax *et al.*,(2003).

One of the most important objectives of this study was to further our understanding of the mechanisms underlying the invasion process of some of the most successful invasive species of the Mediterranean Sea.

In the second chapter, I focused on the morpho-anatomical arsenal that is involved in the adaptation to the novel habitat of both *Siganus* invaders. In the third chapter, we examined the trophic ecology of *Siganus*, in order to determine how the invaders adapt to the resources provided by the novel habitats and how their trophic niche is reshaped during the transition between Red Sea and Mediterranean, within an intra- and inter-specific comparative framework. In the fourth chapter, genetic diversity was monitored in the invading populations from Cyrenaica, and compared with existing data corresponding to an earlier stage of the colonisation and from other areas in the Mediterranean.

5.1. Morphometric variation

Although *S. luridus* and *S. rivulatus* are the most recorded, widespread, and successful invasive fish species in the Mediterranean Sea, and although they have become economically valuable especially in the Cyrenaica Libyan Coast, only little is known about the ecological and evolutionary dynamics that took place in these two species during their invasion of the Cyrenaica coast.

It is worth mentioning that in this study, statistically significant differences were found in the coluration of both *S. luridus* and *S, rivulatus* between the native and the novel habitats. This may be attributed to the impact of the new environment through its difference in environmental factors, and food sources, that differ in their entirety from the native environment. Especially, if taking into consideration the difference in average temperature and salinity rates between the original environments in the Red Sea and the new environments in the Mediterranean, as well as the quality of vegetation in these novel habitats that may be the cause of different colouring between these

species. In particular, dark colouration might help *S. luridus* (normally darker; Shakman *et al.*, 2007) to blend over rocks (possibly to avoid predators), but this might change in novel areas, where more sandy patches might be more frequent. The opposite might occur in *S. rivulatus*.

The use of the geometric morphometrics analysis approach, to study the body shape of these invasive species, revealed a difference between the native habitats as well as the novel habitats where the species were completely separated in the native habitats in terms of body shape, whereas in the novel habitats there was an overlap between all locations from which samples were collected.

The distribution of the data points are more expanded in *S. rivulatus*; it has more spread out variance between data points, which means there is more variance in body shape. *Siganus luridus* has smaller distribution of the data than *S. rivulatus*, which means the *S. luridus* has less shape variation. The PCA showed slight overlap in morphology between the two species. The information of study shows that both *Siganus* species pair follows a different strategy between Cyrenaica habitats and the Red sea habitats. In particular, *S. luridus* and *S. rivulatus* overlap in all study areas coast of Cyrenaica habitats, while they differ considerably with respect to the body shape in the Red sea. Kocovsky *et al.,(*2013) found that the significant morphological differences among *P. flavescens*, he attributed this to genetic differences. While, Abaad *et al.,* (2016) pointed out that the statistically significant differences were observed in body shape between wild-farmed fish and wild fish of *Boops boops* and *Sarpa salpa* as result of feeding used in fish feeds.

The body shape differences between the native and novel habitats are often indicative of adaptation to specific ecological variables. In order to speculate on the cause of patterns in morphology, experiments should be performed to assess the degree of difference of the trophic ecology within *Siganus* species in Cyrenaica coast and the Red sea locations also, the morphological divergence in shape in both *Siganus* species may be caused by hybridization. The results and knowledge about *S. luridus* and *S. rivulatus* can be used to better understand broader aspects of the biology of body shape evolution in other invasive species in the Mediterranean Sea, to determine if the pattern found in *S. luridus* and *S. rivulatus* can be generalized to other invasive species.

5.2. Trophic Ecology

Carbon and nitrogen were used to assess the trophic sources and levels of the *Siganus* species in their novel habitats and compare them to the native environments. The values of carbon in fish

tissues used in *Siganus* species were higher in the Red Sea than in the Cyrenaica habitats, where it is possible to say that these species have used more food sources in the Red Sea than in the Mediterranean Sea. The results showed that the nitrogen values were higher in the Cyrenaica habitats than in the Red Sea, which gives an initial indication that the invasive fish has changed its food level in the Cyrenaica habitats, which may also indicate that these invasive species have wide flexibility in replacing and changing the system of food by sorts and quantity of available food sources.

Despite the large variation in *Siganus* species for δ 13C and δ 15N values between *S. luridus* and *S. rivulatus,* but show no relationship with either total lengt, indicate that differences in the total length for *Siganus* species were not always correlated with δ 15N and δ 13C. In spite of, there were significant differences between the study sites, also between the years; the differences were less significant between species

Stable isotope values of *S. luridus* and *S. rivulatus* varied considerably among Locations in the Cyrenaica coast, when compared to the locations in the Red sea. The two species seem to occupy a border "space" in the new habitats (Cyrenaica coast). From the box plots it seems that both species increase the trophic level when moved into the Cyrenaica coast, while in the Red Sea they appear more tightly clustered and well-segregated.

Lack of isotopic niche overlap between *S. luridus* and *S. rivulatus* in the majority of locations in the Cyrenaica coast indicated a predominantly indirect interaction. I suggest that *Siganus* species induced habitat alteration resulting in a reduction in the dietary niche. Therefore, I attribute any changes in the stable isotope values and population metrics of *S. luridus* and *S. rivulatus* to actual diet alteration in the Cyrenaica coast but, based on δ15N values, I conclude that *Siganus* species are feeding at a similar trophic level in the Red sea, this data supports the conclusion that *Siganus* species are generalist feeders. Once *Siganus* species had become established as the dominant fish species in Cerynaica coast, *Siganus* speciess exhibited change reflecting a shift in diet towards higher trophic levels.

The geometric morphometric analysis proposed that there is considerable niche separation between the two siganids, especially in terms of body shape in native habitats. Stable isotope values of *S. luridus* and *S. rivulatus* varied considerably among locations in the Cyrenaica coast, when compared to the locations in the Red sea, where both species increased the trophic level when moved into the Cyrenaica coast, while in the Red Sea they appear more tightly clustered and

well-segregated. Therefore, I attribute any changes of body shape in the novel habitats for *S. luridus* and *S. rivulatus* to actual diet alteration in the Cyrenaica.

The differences between the diets of *S. luridus* and *S. rivulatus* in these and native and novel habitats may be attributed to the differential quantitative composition of the phytobenthic, as well as regard to the distribution and abundance of benthic algae in the waters of the Cyrenaica coast. Seasonal variations in the abundance and availability of the important phytobenthic of *S. luridus* and *S. rivulatus* could be a major factor leading to variations in the diet of this species in the Cyrenaica coastIt can be concluded that the trophic flexibility of both species should be a key asset for the ability of these species to quickly adapt to novel habitats.

5.3. Genetic analyses

The genetic variation of *S. luridus* and *S. rivulatus* were, predictably, lower than compared to the Red Sea, but not by a large extent, with the haplotype diversity found in the Mediterranean (and especially Cyrenaica) being a good representation of the native diversity, ruling out severe bottlenecks during the colonisation event. A comparison of spatial genetic variation in *S. luridus* and *S. rivulatus* indicated that

In the present study, it was examined mtDNA of *S. luridus* and *S. rivulatus* populations from the Red Sea and Mediterranean Sea. The results have shown based in the mtDNA data higher haplotype diversity (Hd) and higher nucleotide diversity (π), in the Isreal populations in the Red sea (ISR_EIL) than the Cyrenaica population and any other population. While the results of *S. rivulatus* were quite similar, the Haplotype diversity (Hd) were lower. In the Cyrenaica population than the Red sea population, as well as the Nucleotide diversity (π) followed the same trend, with a lows diversity in the Cyrenaica populations than the Red sea population. The data showed quite similar to haplotype diversity (Hd) between Cyrenaica populations and the Red sea populations of *S. luridus*, with a lower nucleotide diversity in the Cyrenaica population than the Red sea population.

Based on the haplotype networks using mtDNA of the *S. luridus* and *S. rivulatus* were well differentiated, did not separate into native population (Red Sea) and Mediterranean population, may reason for the high levels of gene flow between the Red Sea and the Mediterranean Sea. No significant difference of genetic differentiation was observed at mtDNA between the Cyrenaica

population and the Red sea also with all population from the Mediterranean Sea of *Siganus luridus* and *S. rivulatus.*

Inter-population of *S. luridus* the average proportion of nucleotide differences between populations (Kxy), and average number of nucleotide substitutions per site between all *S. luridus* populations (Dxy)

Significant FST and corrected pairwise differences were found between all Cyrenaica population and The Red sea populations also all Mediterranean population. The result of the MDS plot analysis showed differences between populations of *S. luridus* according to mtDNA. Significant F_{ST} and corrected pairwise differences were found between all Cyrenaica population and the Red sea populations also all Mediterranean population. The F_{ST} values were not significantly different between Cyrenaica populations and the Red sea populations of *S. rivulatus.* This study gave a quick overview of the levels of genetic variation resulting from the adaptation process that happened to these species in the new environments. These results confirm that these species were not isolated from the source, and that the flow of these species is still continuous so that there has been no genetic asphyxia or geographical isolation of these species in their new environment.

5.4. The Invasive Species and their impact on Biodiversity in the Cyrenaica coast

As is well known, the monitoring and control of invasive species is difficult in terms of determining the time of invasion as well as the population size and distribution of the invaders, especially in marine environments because these environments are open, and the impact of these invasions is unpredictable.

Large areas of the coast of Cyrenaica have been subjected to the invasion of species that will change the biodiversity of this region of the Mediterranean Sea, with predicted loss of biodiversity and the degradation of the ecosystem and its functions (Sghaier *et al.*, 2013). The Cyrenaica coast is often referred to by the Global Environment Fund as one of the last ten gardens located in the Mediterranean Sea (Otero *et al.*,2013) where no more than 2 million people are located, with the absence of major local human impacts, the marine environment in this region is primarily threatened by biological invasions.

This is particularly worrying, because there is no database on biodiversity or invasive and exotic species in this part of the Mediterranean, due to the lack of field studies and scientific

research in this field. In this context, every effort to increase our understanding of the mechanisms (i.e. behavioural, ecological, evolutionary…) of settlement will go some way to empower conservation agencies and policy makers with some evidence to support management strategies. With my study, we were able to detect a relaxed morphospace and a remarkable trophic flexibility in *S. luridus* and *S. rivulatus*, bith of which are features of a successful invader. The more blurred morphological boundaries, and the greater chromatic heterogeneity in Mediterranean rabbitfish populations may indicate some potential for hybridisation, which is a phenomenon known to occur in *Siganus* species (Kuriiwa *et al.,* 2007) and compatible with a demographic scenario of invasion (Yamamichi and Tand ,2014); and this will be an interesting question to address.

Finally, the genetic structure of *S. rivulatus* (and its presently more restricted distribution in the Medieterranean) appears consistent with a slightly lower dispersal propensity and perhaps a smaller effective population size, given this species' longer time since invasion compared to *S. luridus* (in Libya the first record for *S. rivulatus* dates back to 1927, while for *S. luridus* it is more recent, 1956). However, more targeted studies will be required to explain the factors underlying differences in dispersal and connectivity between these species.

5.5. Future Studies

Despite the use of different approaches and methods in this study, many questions have emerged which should be examined and dealt with in future studies.

The more heterogeneous colouring pattern in the novel habitat deserves further investigations, with either experimental/observational studies, or genetic inference. Several hypotheses can be formulated in this context, including the adaptation to blend into different substrates, and the possibility that recombination resulting from hybridisation may generate novel phenotypes (Seehausen, 2004).

Among the studies that can be derived from the results of this study is a study on the competition between invasive species and native species on food sources in new environments. Especially since there are some studies indicating the existence of competition between *Siganus* species and *Sarpa salpa* on food resources. That can be inferred through the use of traditional methods, such as stomach content analysis, possibly aided by DNA metabarcoding to achieve greater resolution; or a tailored stable isotope approach, ideally including more species, at various trophic levels, in order to track changes in the trophic web structure as a whole.

Perhaps, with a clear vision of the whole food web, it may become possible to predict whether native species can eliminated/outcompeted by the invasive species and the ripple effect that this may have on the ecosystem, and the services provided, such as coastal fisheries.

In terms of genetic investigations, it is paramount to test the hypothesis of hybridization between these invasive species, and in particular, i) whether it occurs at all; ii) if so, how frequent it is, iii) whether there is a preferential directionality (i.e. which species is more likely to be maternal); iv) whether any advantageous trait may result from the mixing of the genomes.

In general, such future studies, which are essential for managing ecosystems in proactive and adaptive way, would give a more comprehensive idea of biodiversity trends and their direction, and the extent of the degradation caused by the introduction of exotic species. Thus, we have contributed to shedding light on the importance of biodiversity and the dangers of these invasive species in the coast of Cyrenaica habitats and alerting the decision makers with the importance of such research.

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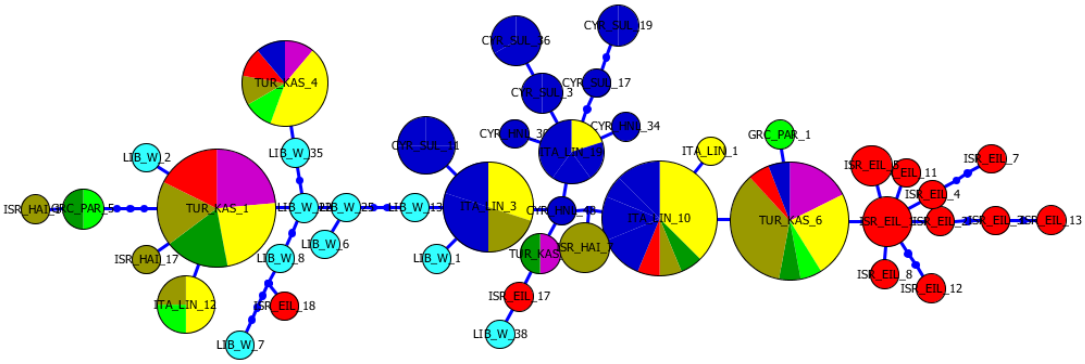
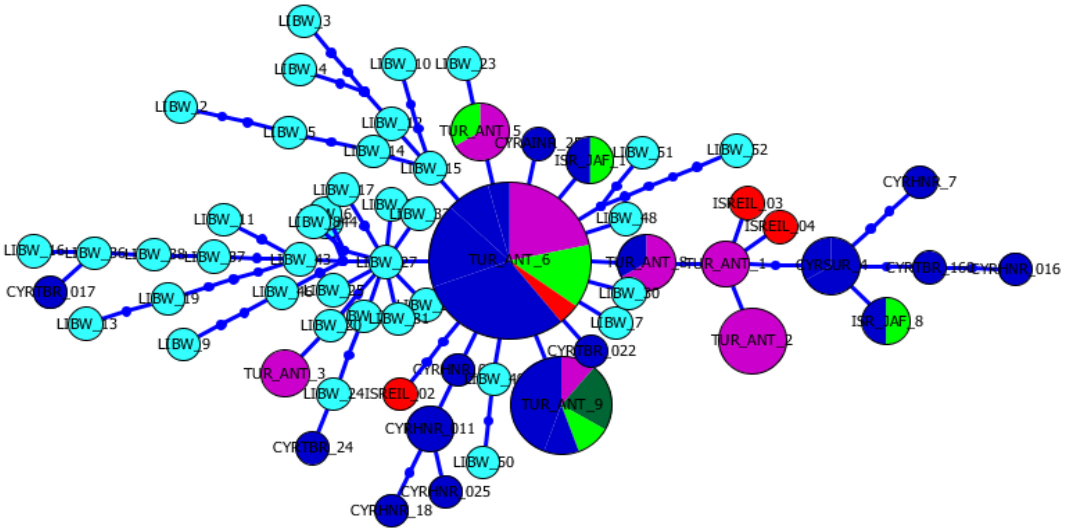
7. Appendix

7.1. Haplotype network of *S. luridus* that collected from Cyrenaica coast and the GenBank with the Sequences from Shakman *et al.*, (2008)



7.2. Haplotype network of *S. rivulatus* that collected from Cyrenaica coast and the GenBank with the sequences from Shakman *et al.*, (2008)



7.3. The last updating the list of marine invasive species in the Libyan waters.

Seriola rivoliana (Shakman *et al*., 2017)

Seriola fasciata (Shakman *et al.*, 2017)

Sphoeroides pachygaster (Shakman *et al* ., 2017)

Etrumeus golanii, (Shakman *et al.*, 2017)

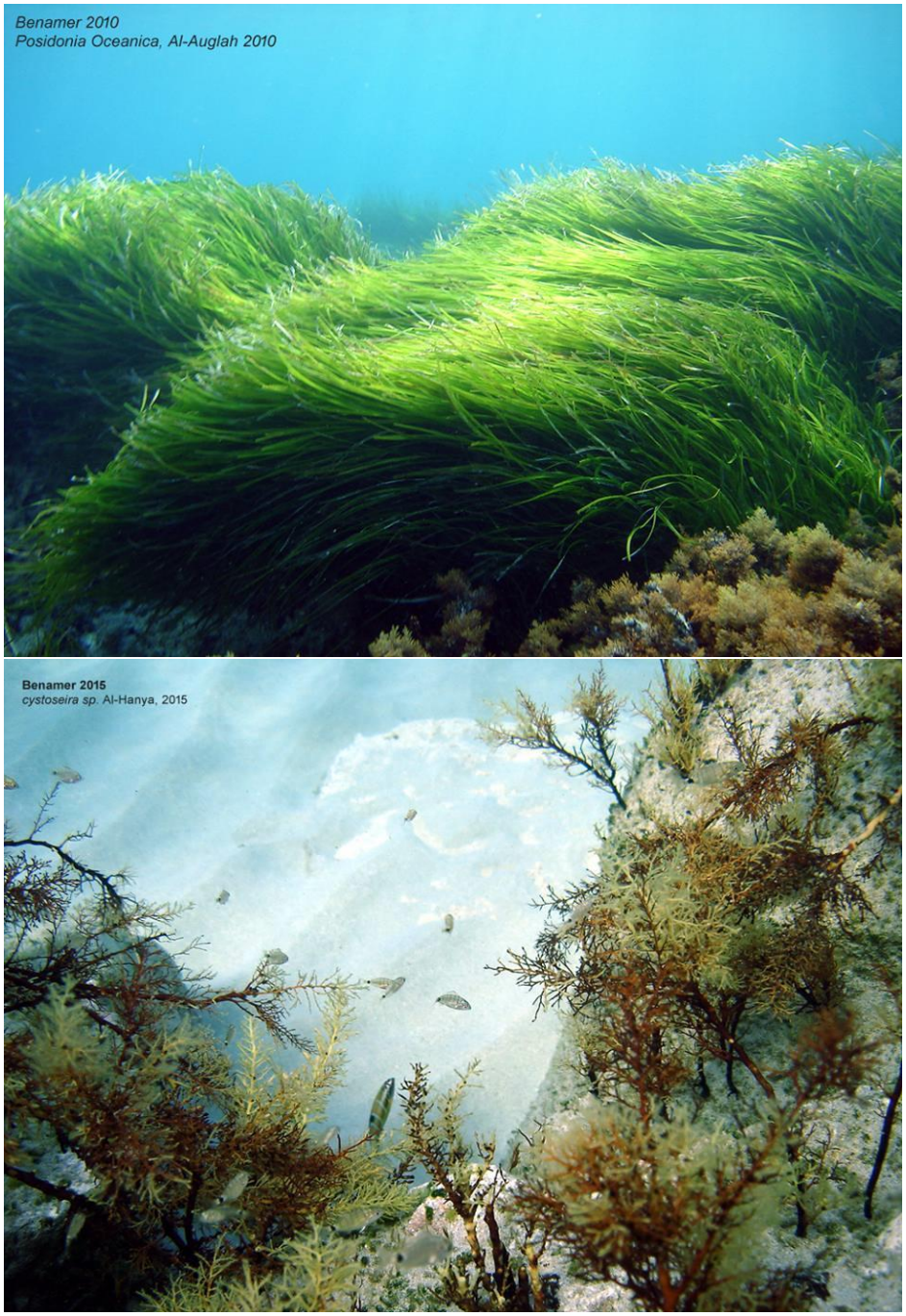
Portunus segnis, (Shakman *et al* ., 2017)

Sepioteuthis lessoniana (Shakman *et al* ., 2017)

Galeocerdo cuvier , (Tobuni *et al* ., 2017)

Caulerpa taxifolia, (Shakman *et al* ., 2017)

7.4. Algae, seaweed marine coastline background, Cyrenaica Sea.



7.5. Course about the Survivor's Guide Stable Isotope Ecology in Sicily.

SCIE CENTER SIRACUSA
Sicily Center for International Education

This is to certify that
ABDULGHANI A. HAMAD ABDULGHANI
Attended the course:

The Survivor's Guide to Stable Isotope Ecology

Held by:
Prof. Chris Harrod, Universidad de Antofagasta, Chile
Prof. Andrew Jackson, Trinity College Dublin
Prof. Seth Newsome, University of New Mexico, USA
Dr. Brian Hayden, Science Manager, Stable Isotopes in Nature Laboratory, University of New Brunswick, Canada
Dr. Alexia Massa-Gallucci, SCIE Center, Italy

At the
Sicily Center for International Education
Siracusa, Sicily, Italy
2-8 April 2017

Alexia Massa-Gallucci
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Susi Kimbell
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7.6. The online course Analysis of Organismal Form at the University of Manchester

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13 January 2016

Dear Mr Abdulghani Abdulghani

I am writing to confirm that you have successfully participated in the online course *Analysis of Organismal Form* (course code BIOL51111) in the first semester of the 2015-16 academic year (9 November to 11 December 2015).

The course is intended to provide a general introduction to the methods of geometric morphometrics and examples of their application in various biological disciplines. The workload of the course is equivalent to 48 hours of lecture and practical classes.

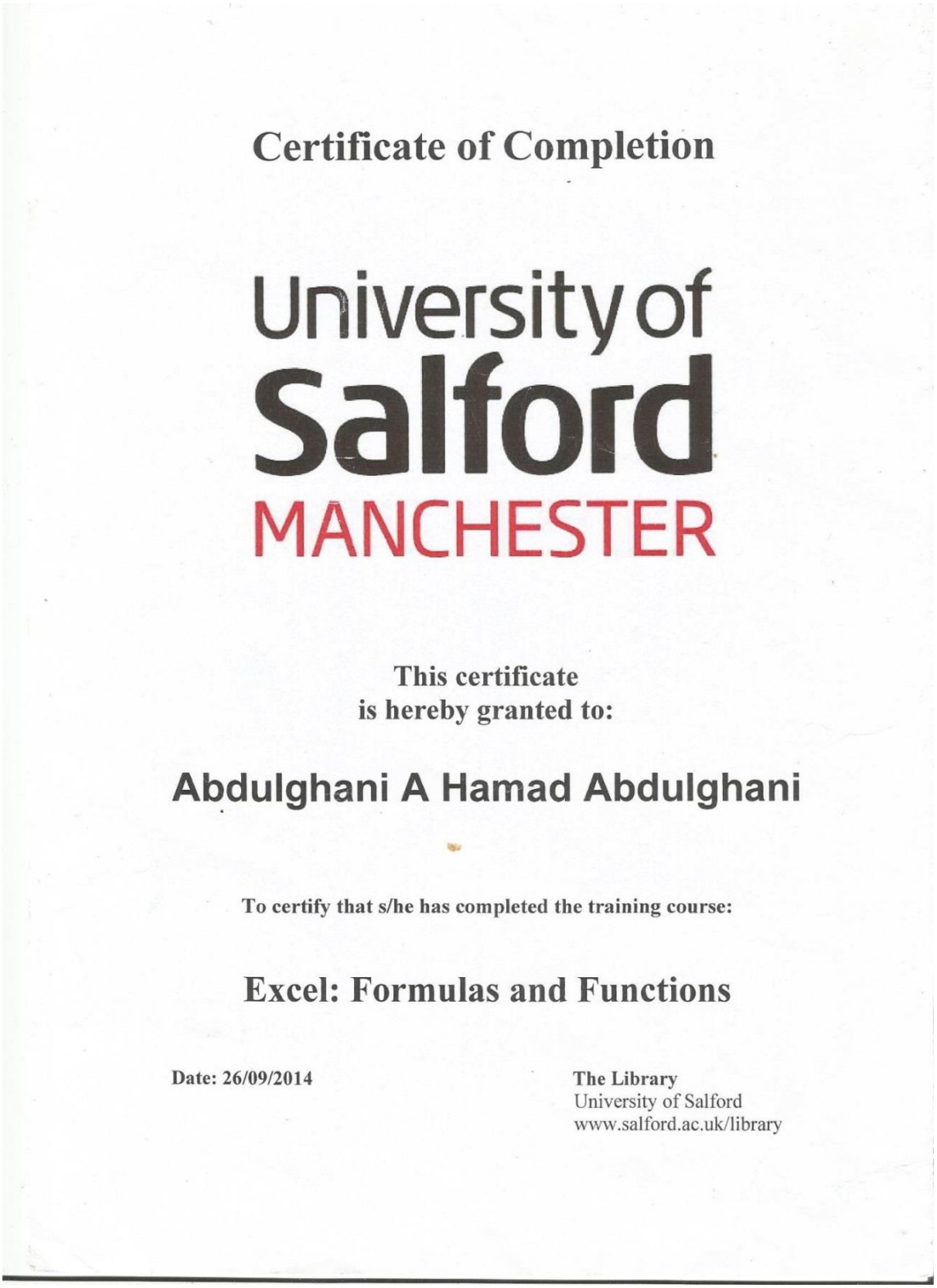
Other than this confirmation of your participation, the University does not provide any formal assessment or certification for external participants of this course.

I hope that the course was useful for you and I wish you success in applying morphometric approaches to your research.

Yours sincerely

Dr Christian Peter Klingenberg

7.7. Training course on Excel: Formulas and Functions at University of Salford



Certificate of Completion

University of Salford MANCHESTER

This certificate
is hereby granted to:

Abdulghani A Hamad Abdulghani

To certify that s/he has completed the training course:

Excel: Formulas and Functions

Date: 26/09/2014

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